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(54) Title: OLIGOMERS FOR MODULATING VIRAL PROCESSES

(57) Abstract

The present invention provides oligomers useful for modulating viral processes such as cytomegalovirus and papillomaviruses. The oligomers are comprised of subunits, at least one of which is a protein nucleic acid subunit. Therapeutic and diagnostic methods are also provided.

OLIGOMERS FOR MODULATING VIRAL PROCESSES

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Serial No. 08/104,438 filed August 9, 1993 which is a a continuation-in-part of 08/009,263 filed January 25, 1993 which is a continuation-in-part of PCT/US91/05815 filed August 14, 1991 which is a continuation-in-part of U.S. Serial No. 568,366 filed August 16, 1990. This application is also a continuation-in-part of PCT/US93/03075 filed March 31, 1993 which is a continuation-in-part of U.S. Serial No. 860,925 filed March 31, 1992 which is a continuation-in-part of U.S. Serial No. 835,946 filed March 3, 1992 which is a continuation-in-part of U.S. Serial No. 445,196 filed December 4, 1989. These applications are assigned to the assignee of this invention. The entire disclosure of each is incorporated herein by reference.

FIELD OF THE INVENTION

This invention is directed to compounds that are not polynucleotides yet which bind in a complementary fashion to DNA and RNA strands. In particular, the invention concerns compounds wherein naturally-occurring nucleobases or other nucleobase-binding moieties are covalently bound to a polyamide backbone. These compounds are useful for therapeutic and other applications directed to modulating viral processes.

BACKGROUND OF THE INVENTION

Peptide Nucleic Acids (PNAs)

Genes function by transferring information to a messenger RNA (mRNA) molecule, a process referred to as transcription. The interaction of mRNA with the ribosomal complex directs the synthesis of a protein encoded within its sequence. This synthetic process is known as translation and requires the presence of various co-factors and building blocks, the amino acids, and their transfer RNAs (tRNA), all of which are present in normal cells.

The initiation of transcription requires specific recognition of a promoter DNA sequence by the RNA-synthesizing enzyme, RNA polymerase. In many cases in prokaryotic cells, and most likely in all cases in eukaryotic cells, this recognition is preceded by sequence-specific binding of protein transcription factors to the promoter. Other proteins which bind to the promoter, but whose binding prohibits action of RNA polymerase, are known as repressors. Thus, gene activation is typically regulated positively by transcription factors and negatively by repressors.

Most conventional drugs function by interaction with and modulation of one or more targeted endogenous proteins, *e.g.*, enzymes. However, such drugs are typically not specific for targeted proteins but interact with other proteins as well. Thus, a relatively large dose of drug must be used to effectively modulate a targeted protein. Typical

daily doses of drugs are from 10^{-5}-10^{-1} millimoles per kilogram of body weight or 10^{-3}-10 millimoles for a 100 kilogram person. If this modulation could instead be effected by interaction with and inactivation of mRNA, a dramatic reduction in the necessary amount of drug could likely be achieved, along with a corresponding reduction in adverse side effects. Further reductions could be achieved if such interaction could be rendered site-specific. Given that a functioning gene continually produces mRNA throughout the life of the cell, it would thus be even more advantageous if gene transcription could be arrested in its entirety.

Oligodeoxynucleotides offer such opportunities. For example, synthetic oligodeoxynucleotides have been used as antisense probes to block and eventually lead to the breakdown of mRNA. It also may be possible to modulate the genome of an animal by, for example, triple helix formation using oligonucleotides or other DNA recognizing agents. However, there are a number of drawbacks associated with triple helix formation. For example, it can only be used for homopurine sequences and it requires unphysiologically high ionic strength and low pH.

Unmodified oligonucleotides are impractical both in the antisense approach and in the triple helix approach because they have short *in vivo* half-lives. They are also poor penetrators of the cell membrane.

These problems have resulted in an extensive search for improvements and alternatives. For example, the problems arising in connection with double-stranded DNA (dsDNA) recognition through triple helix formation have been diminished by a clever "switch back" chemical linking whereby a sequence of polypurine on one strand is recognized, and by "switching back", a homopurine sequence on the other strand can be recognized. Also, competent helix formation has been obtained by using artificial bases, thereby improving binding conditions with regard to ionic strength and pH.

In order to improve half life as well as membrane penetration, a large number of variations in polynucleotide

backbones has been undertaken. These variations include the use of methylphosphonates, monothiophosphates, dithiophosphates, phosphoramidates, phosphate esters, bridged phosphoro-amidates, bridged phosphorothioates, bridged methylene-phosphonates, dephospho internucleotide analogs with siloxane bridges, carbonate bridges, carboxymethyl ester bridges, acetamide bridges, carbamate bridges, thioether, sulfoxy, sulfono bridges, various "plastic" DNAs, α-anomeric bridges, and borane derivatives.

The great majority of these modifications has led to decreased stability for hybrids formed between the modified oligonucleotide and its complementary, native oligonucleotide, as assayed by measuring T_m values. Consequently, it is generally understood in the art that backbone modifications destabilize such hybrids, *i.e.*, result in lower T_m values, and should be kept to a minimum.

In WO 92/20702, moieties denominated peptide nucleic acids (PNAs) are disclosed wherein ligands are linked to a polyamide backbone through aza nitrogen atoms. In PCT/IB94/ 00142 filed April 25, 1994 peptide nucleic acids are disclosed in which their recognition moieties are linked to the polyamide backbone additionally through amido and/or ureido tethers. PCT/EP 92/01219 filed May 22, 1992 also discloses protein nucleic acids.

These peptide nucleic acids are synthesized by adaptation of certain peptide synthesis procedures, either in solution or on a solid phase. The synthons used are certain monomer amino acids or their activated derivatives, protected by standard groups. These oligonucleotide analogs also can be synthesized by using the corresponding diacids and diamines.

Peptide nucleic acid oligomers have been found to be superior to prior reagents in that they have significantly higher affinity for complementary single stranded DNA (ssDNA). These compounds are also able to form triple helices wherein a first PNA strand binds with RNA or ssDNA and a second PNA strand binds with the resulting double helix

or with the first PNA strand. PNAs generally possess no significant charge and are water soluble, which facilitates cellular uptake. Moreover, PNAs contain amides of non-biological amino acids, making them biostable and resistant to enzymatic degradation, for example, by proteases.

Accordingly, PNAs can ideally be used to target RNA and ssDNA to produce antisense-type gene regulating moieties. Reagents that bind sequence-specifically to dsDNA, RNA, or ssDNA have applications as gene targeted drugs useful for modulating viral processes. PNAs can also be useful in diagnostics, as for example, as probes for specific mRNAs.

Cytomegalovirus (CMV)

CMV's are ubiquitous in nature and are the most common causes of intrauterine infection. Congenital infection is common in newborns of infected mothers. In some populations, as much as 10% of children display perinatal infections. In a small percentage of newborns, the infection is virulent, involving multiple organs. Pronounced involvement of the reticuloendothelial and central nervous system is typical; and the infection is a major cause of mental retardation. Careful testing demonstrates that as many as 50% of severely, prenatally infected adults may display neuropsychiatric disease or deafness. Although extraneural organs are usually spared chronic morbidity, the virus can be detected in the kidney for years.

In the adult, cytomegalovirus-induced mononucleosis is a lingering illness that causes significant morbidity. If it occurs in immunosuppressed patients, the disease is more severe, and it may be complicated by other infectious pathogens which may be fatal. Cytomegalovirus retinitis is a severe problem in immunosuppressed patients that often leads to blindness. Immunosuppressed patients are also very susceptible to CMV pneumonitis, which is one of the most lethal of human viral diseases. Although cytomegalovirus may play a role in the progression of HIV infection to AIDS by stimulating the transcription of the HIV long terminal

repeats (LTR) in non-transformed co-infected T cells, histologic examination of adrenals and brains from AIDS patients has suggested that the adrenalitis, encephalitis and peripheral neuropathy were caused by CMV infection.

CMV is considered to be an oncogenic virus. *In vitro*, CMV can transform cells and stimulate growth. Both human and non-human cells can undergo transformation when incubated with CMV. Transformed cells contain CMV antigens that are oncogenic when inoculated into appropriate animals. Moreover, oncogenic potential has been associated with specific segments of the CMV genome.

Human CMV is a large, enveloped virus whose genome consists of a double-stranded DNA molecule which is approximately 240,000 nucleotides in length. This genome is the most complex of all DNA viruses and is approximately 50% larger than the genome of herpes simplex virus (HSV). Intact viral DNA is composed of contiguous long (L) and short (S) segments, each of which contains regions of unique DNA sequence flanked by homologous regions of repetitive sequence. As a group, the human CMV isolates share at least 80% sequence homology, making it nearly impossible to classify cytomegaloviruses into subgroups or subtypes, although variations in the restriction endonuclease patterns of various CMV DNA preparations are identifiable in epidemiologically unrelated strains. The DNA of the prototypic strain of CMV (AD 169) has been sequenced and reported to contain a conservative estimate of 175 unique translational open reading frames (ORFs). At least 42 ORFs encode putative glycoproteins and several of the CMV ORFs putatively encode proteins with amino acid homology to human opsin receptor proteins.

Effective therapy for CMV has not yet been developed despite studies on a number of antivirals. Interferon, transfer factor, adenine arabinoside (Ara-A), acycloguanosine (Acyclovir, ACV) and certain combinations of these drugs have been ineffective in controlling CMV infection. Based on preclinical and clinical data, foscarnet

(PFA) and ganciclovir (DHPG) show limited potential as antiviral agents. PFA treatment has resulted in the resolution of CMV retinitis in five AIDS patients. DHPG studies have shown efficacy against CMV retinitis or colitis. DHPG seems to be well tolerated by treated individuals, but the appearance of a reversible neutropenia, the emergence of resistant strains of CMV upon long-term administration, and the lack of efficacy against CMV pneumonitis limit the long term applications of this compound. The development of more effective and less-toxic therapeutic compounds and methods is needed for both acute and chronic use.

Classical therapeutics has generally focused upon interactions with proteins in efforts to moderate their disease causing or disease potentiating functions. Such therapeutic approaches have failed for cytomegalovirus infections. The present invention is directed to an alternative approach to the treatment of such infections, the inhibition of cytomegalovirus gene expression through the mediation of oligomers comprising PNA.

Papillomaviruses

The papillomaviruses (PV) are widespread in nature and are generally associated with benign epithelial and fibroepithelial lesions commonly referred to as warts. They have been detected in and isolated from a variety of higher vertebrates including human, cattle, rabbits, deer and several avian species. Although these viruses are generally associated with benign lesions, a specific subset of the viruses have been associated with lesions that may progress to carcinomas. The implication that these viruses may play an etiologic role in the development of some human cancers follows from numerous studies that have shown the presence of transcriptionally active human papillomavirus (HPV) deoxyribonucleic acids in a high percentage of certain cancerous lesions. Zur Hausen, H. and Schneider, A. 1987. In: The Papovaviridae, vol. 2, edited by N. P. Salzman and P. M. Howley, pp. 245-264. Plenum Press, New York.

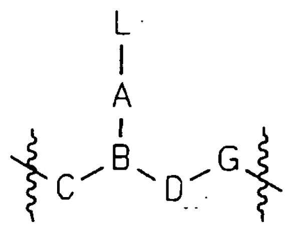
In man, human papillomaviruses cause a variety of disease including common warts of the hands and feet, laryngeal warts and genital warts. More than 57 types of HPV have been identified so far. Each HPV type has a preferred anatomical site of infection; each virus can generally be associated with a specific lesion. Genital warts, also referred to as venereal warts and condylomata acuminata, are one of the most serious manifestations of PV infection. As reported by the Center for Disease Control, the sexual mode of transmission of genital warts is well established and the incidence of genital warts is on the increase. The seriousness of genital warts is underlined by the recent discovery that HPV DNA can be found in all grades of cervical intraepithelial neoplasia (CIN I-III) and that a specific subset of HPV types can be found in carcinoma *in situ* of the cervix. Consequently, women with genital warts, containing specific HPV types are now considered at high risk for the development of cervical cancer. Current treatments for genital warts are inadequate. Thus, the provision of oligomer therapies for papillomavirus infections in accordance with this invention satisfies the long-felt need for such therapies.

SUMMARY OF THE INVENTION

The present invention provides oligomers comprising peptide nucleic acids (PNAs), that bind complementary ssDNA and RNA strands through their oligoribonucleotide ligands which are linked to a peptide backbone. The sequence of the oligoribonucleotide ligands specifies the target to which they bind. These PNAs are extremely useful drugs for treating diseases like cancer, AIDS and other viral infections. These compositions are also useful in diagnostic applications and as research tools.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Oligomers of the present invention include at least least one subunit that is a peptide nucleic acid subunit of the formula:



(I)

wherein:

L is one of the adenine, thymine, cytosine or guanine heterocyclic bases of the oligomer;

C is $(CR^6R^7)_y$ where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently selected from the group consisting of hydrogen, (C_2-C_6)alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C_1-C_6)alkoxy, (C_1-C_6)alkylthio, NR^3R^4 and SR^5, where each of R^3 and R^4 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl, hydroxy- or alkoxy- or alkylthio-substituted (C_1-C_4)alkyl, hydroxy, alkoxy, alkylthio and amino; and R^5 is hydrogen, (C_1-C_6)alkyl, hydroxy-, alkoxy-, or alkylthio-substituted (C_1-C_6)alkyl, or R^6 and R^7 taken together complete an alicyclic or heterocyclic system;

D is $(CR^6R^7)_z$ where R^6 and R^7 are as defined above;

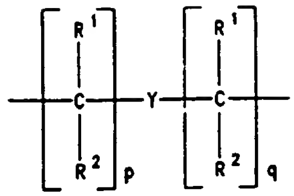
each of y and z is zero or an integer from 1 to 10, the sum y + z being greater than 2 but not more than 10;

G is $-NR^3CO-$, $-NR^3CS-$, $-NR^3SO-$ or $-NR^3SO_2-$, in either orientation, where R^3 is as defined above;

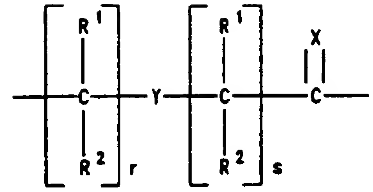
each pair of A and B is selected such that:

(a) A is a group of formula (IIa), (IIb) or (IIc) and B is N or R^3N^+; or

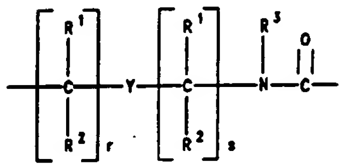
(b) A is a group of formula (IId) and B is CH;



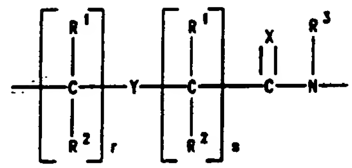
(IIa)



(IIb)



(IIc)



(IId)

where:

X is O, S, Se, NR^3, CH_2 or $C(CH_3)_2$;

Y is a single bond, O, S or NR^4;

each of p and q is zero or an integer from 1 to 5, the sum p+q being not more than 10;

each of r and s is zero or an integer from 1 to 5, the sum r+s being not more than 10;

each R^1 and R^2 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl which may be hydroxy- or alkoxy- or alkylthio-substituted, hydroxy, alkoxy, alkylthio, amino and halogen.

Subunits, as used herein, refers to basic unit which are chemically similar and which can form polymers. Repeating basic units form polymers referred to as "oligomers". Oligomers of the present invention may thus refer to oligomers in which substantially all subunits of the oligomer are subunits as described in Formula I. Oligomers of the present invention may also comprise one or more subunits which are naturally occuring nucleotides or nucleotide analogs as long as at least one subunit satisfies Formula I. Thus, oligomers as used herein may refer to a range of oligomers from oligomers comprising only one PNA

subunit as defined in Formula I to oligomers in which every subunit is a PNA subunit as defined in Formula I.

Those subunits which are not PNA subunits comprise naturally occuring bases, sugars, and intersugar (backbone) linkages as well as non-naturally occurring portions which function similarly to naturally occurring portions.

Sequences of oligomers of the present invention are defined by reference to the L group (for PNA subunits) or nucleobase (for nucleotide subunits) at a given position. Thus, for a given oligomer, the nomeclature is modeled after traditional nucleotide nomenclature, identifying each PNA subunit by the identity of its L group such as the heterocycles adenine (A), thymine (T), guanine (G) and cytosine (C) and identifying nucleotides or nucleosides by these same heterocycle residing on the sugar backbone. The sequences are conveniently provided in traditional 5' to 3' or amino to carboxy orientation.

Oligomers of the present invention may range in size from about 5 to about 50 subunits in length. In other embodiments of the present invention, oligomers may range in size from about 10 to about 30 subunits in length. In still other embodiments of the present invention oligomers may range in size from about 10 to about 25 subunits in length. In yet further embodiments of the present invention, oligomers may range in size from about 12 to about 20 subunits in length.

The preparation of protein nucleic acid oligomers is known in the art, such as is described in PCT/EP 92/01219 filed May 22, 1992, which is incorporated by reference herein in its entirety.

Briefly, the principle of anchoring molecules onto a solid matrix, which helps in accounting for intermediate products during chemical transformations, is known as Solid-Phase Synthesis or Merrifield Synthesis (*see, e.g.*, Merrifield, *J. Am. Chem. Soc.*, **1963**, *85*, 2149 and *Science*, **1986**, *232*, 341). Established methods for the stepwise or fragmentwise solid-phase assembly of amino acids into

peptides normally employ a beaded matrix of slightly cross-linked styrene-divinylbenzene copolymer, the cross-linked copolymer having been formed by the pearl polymerization of styrene monomer to which has been added a mixture of divinylbenzenes. A level of 1-2% cross-linking is usually employed. Such a matrix also can be used in solid-phase PNA synthesis in accordance with the present invention.

Concerning the initial functionalization of the solid phase, more than fifty methods have been described in connection with traditional solid-phase peptide synthesis (*see, e.g.*, Barany and Merrifield in "The Peptides" Vol. 2, Academic Press, New York, 1979, pp. 1-284, and Stewart and Young, "Solid Phase Peptide Synthesis", 2nd Ed., Pierce Chemical Company, Illinois, 1984). Reactions for the introduction of chloromethyl functionality (Merrifield resin; via a chloromethyl methyl ether/$SnCl_4$ reaction), aminomethyl functionality (via an N-hydroxymethylphthalimide reaction; *see*, Mitchell, *et al.*, *Tetrahedron Lett.*, **1976**, *3795*), and benzhydrylamino functionality (Pietta, *et al.*, *J. Chem. Soc.*, **1970**, 650) are the most widely applied. Regardless of its nature, the purpose of the functionality is normally to form an anchoring linkage between the copolymer solid support and the C-terminus of the first amino acid to be coupled to the solid support. As will be recognized, anchoring linkages also can be formed between the solid support and the amino acid N-terminus. It is generally convenient to express the "concentration" of a functional group in terms of millimoles per gram (mmol/g). Other reactive functionalities which have been initially introduced include 4-methylbenzhydrylamino and 4-methoxybenzhydrylamino. All of these established methods are in principle useful within the context of the present invention. Preferred methods for PNA synthesis employ aminomethyl as the initial functionality, in that aminomethyl is particularly advantageous with respect to the incorporation of "spacer" or "handle" groups, owing to the reactivity of the amino group of the aminomethyl functionality with respect to the essentially quantitative

formation of amide bonds to a carboxylic acid group at one end of the spacer-forming reagent. A vast number of relevant spacer- or handle-forming bifunctional reagents have been described (*see*, Barany, *et al.*, *Int. J. Peptide Protein Res.*, **1987**, *30*, 705), especially reagents which are reactive towards amino groups such as found in the aminomethyl function. Representative bifunctional reagents include 4-(haloalkyl)aryl-lower alkanoic acids such as 4-(bromomethyl)phenylacetic acid, Boc-aminoacyl-4-(oxymethyl)aryl-lower alkanoic acids such as Boc-aminoacyl-4-(oxymethyl)phenylacetic acid, N-Boc-p-acylbenzhydrylamines such as N-Boc-p-glutaroylbenzhydrylamine, N-Boc-4'-lower alkyl-p-acylbenzhydrylamines such as N-Boc-4'-methyl-p-glutaroylbenzhydrylamine, N-Boc-4'-lower alkoxy-p-acylbenzhydrylamines such as N-Boc-4'-methoxy-p-glutaroyl-benzhydrylamine, and 4-hydroxymethylphenoxyacetic acid. One type of spacer group particularly relevant within the context of the present invention is the phenylacetamidomethyl (Pam) handle (Mitchell and Merrifield, *J. Org. Chem.*, **1976**, *41*, 2015) which, deriving from the electron withdrawing effect of the 4-phenylacetamidomethyl group, is about 100 times more stable than the classical benzyl ester linkage towards the Boc-amino deprotection reagent trifluoroacetic acid (TFA).

Certain functionalities (*e.g.*, benzhydrylamino, 4-methylbenzhydrylamino and 4-methoxybenzhydrylamino) which may be incorporated for the purpose of cleavage of a synthesized PNA chain from the solid support such that the C-terminal of the PNA chain is in amide form, require no introduction of a spacer group. Any such functionality may advantageously be employed in the context of the present invention.

An alternative strategy concerning the introduction of spacer or handle groups is the so-called "preformed handle" strategy (*see*, Tam, *et al.*, *Synthesis*, **1979**, 955-957), which offers complete control over coupling of the first amino acid, and excludes the possibility of complications arising from the presence of undesired functional groups not related to the peptide or PNA

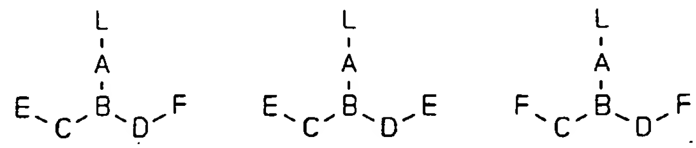
synthesis. In this strategy, spacer or handle groups, of the same type as described above, are reacted with the first amino acid desired to be bound to the solid support, the amino acid being N-protected and optionally protected at the other side-chains which are not relevant with respect to the growth of the desired PNA chain. Thus, in those cases in which a spacer or handle group is desirable, the first amino acid to be coupled to the solid support can either be coupled to the free reactive end of a spacer group which has been bound to the initially introduced functionality (for example, an aminomethyl group) or can be reacted with the spacer-forming reagent. The space-forming reagent is then reacted with the initially introduced functionality. Other useful anchoring schemes include the "multidetachable" resins (Tam, *et al.*, *Tetrahedron Lett.*, **1979**, 4935 and *J. Am. Chem. Soc.*, **1980**, *102*, 611; Tam, *J. Org. Chem.*, **1985**, *50*, 5291), which provide more than one mode of release and thereby allow more flexibility in synthetic design.

Suitable choices for N-protection are the tert-butyloxycarbonyl (Boc) group (Carpino, *J. Am. Chem. Soc.*, **1957**, *79*, 4427; McKay, *et al.*, *J. Am. Chem. Soc.*, **1957**, *79*, 4686; Anderson, *et al.*, *J. Am. Chem. Soc.*, **1957**, *79*, 6180) normally in combination with benzyl-based groups for the protection of side chains, and the 9-fluorenylmethyloxy-carbonyl (Fmoc) group (Carpino, *et al.*, *J. Am. Chem. Soc.*, **1970**, *92*, 5748 and *J. Org. Chem.*, **1972**, *37*, 3404), normally in combination with tert-butyl (tBu) for the protection of any side chains, although a number of other possibilities exist which are well known in conventional solid-phase peptide synthesis. Thus, a wide range of other useful amino protecting groups exist, some of which are Adoc (Hass, *et al.*, *J. Am. Chem. Soc.*, **1966**, *88*, 1988), Bpoc (Sieber, *Helv. Chem. Acta.*, **1968**, *51*, 614), Mcb (Brady, *et al.*, *J. Org. Chem.*, **1977**, *42*, 143), Bic (Kemp, *et al.*, *Tetrahedron*, **1975**, 4624), the o-nitrophenylsulfenyl (Nps) (Zervas, *et al.*, *J. Am. Chem. Soc.*, **1963**, *85*, 3660), and the dithiasuccinoyl (Dts) (Barany, *et al.*, *J. Am. Chem. Soc.*, **1977**, *99*, 7363).

These amino protecting groups, particularly those based on the widely-used urethane functionality, successfully prohibit racemization (mediated by tautomerization of the readily formed oxazolinone (azlactone) intermediates (Goodman, *et al.*, *J. Am. Chem. Soc.*, **1964**, *86*, 2918)) during the coupling of most α-amino acids. In addition to such amino protecting groups,a whole range of otherwise "worthless" nonurethane-type of amino protecting groups are applicable when assembling PNA molecules, especially those built from achiral units. Thus, not only the above-mentioned amino protecting groups (or those derived from any of these groups) are useful within the context of the present invention, but virtually any amino protecting group which largely fulfills the following requirements: (1) stability to mild acids (not significantly attacked by carboxyl groups); (2) stability to mild bases or nucleophiles (not significantly attacked by the amino group in question); (3) resistance to acylation (not significantly attacked by activated amino acids). Additionally: (4) the protecting group must be close to quantitatively removable, without serious side reactions, and (5) the optical integrity, if any, of the incoming amino acid should preferably be highly preserved upon coupling. Finally, the choice of side-chain protecting groups, in general, depends on the choice of the amino protecting group, since the protection of side-chain functionalities must withstand the conditions of the repeated amino deprotection cycles. This is true whether the overall strategy for chemically assembling PNA molecules relies on, for example, differential acid stability of amino and side-chain protecting groups (such as is the case for the above-mentioned "Boc-benzyl" approach) or employs an orthogonal, that is, chemoselective, protection scheme (such as is the case for the above-mentioned "Fmoc-tBu" approach),

Following coupling of the first amino acid, the next stage of solid-phase synthesis is the systematic elaboration of the desired PNA chain to incorporate additional subunits using monomer synthons. Novel monomer

synthons may be selected from the group consisting of amino acids, diacids and diamines having general formulae:



(II) (III) (IV)

wherein L, A, B, C and D are as defined above, except that any amino groups therein may be protected by amino protecting groups; E is COOH, CSOH, SOOH, SO_2OH or an activated derivative thereof; and F is NHR^3 or $NPgR^3$, where R^3 is as defined above and Pg is an amino protecting group. This elaboration involves repeated deprotection/coupling cycles. The temporary protecting group, such as a Boc or Fmoc group, on the last-coupled amino acid is quantitatively removed by a suitable treatment, for example, by acidolysis, such as with trifluoroacetic acid, in the case of Boc, or by base treatment, such as with piperidine, in the case of Fmoc, so as to liberate the N-terminal amine function.

The next desired N-protected amino acid is then coupled to the N-terminal of the last-coupled amino acid. This coupling of the C-terminal of an amino acid with the N-terminal of the last-coupled amino acid can be achieved in several ways. For example, it can be bound by providing the incoming amino acid in a form with the carboxyl group activated by any of several methods, including the initial formation of an active ester derivative such as a 2,4,5-trichlorophenyl ester (Pless, *et al.*, *Helv. Chim. Acta*, **1963**, *46*, 1609), a phthalimido ester (Nefkens, *et al.*, *J. Am. Chem. Soc.*, **1961**, *83*, 1263), a pentachlorophenyl ester (Kupryszewski, *Rocz. Chem.*, **1961**, *35*, 595), a pentafluorophenyl ester (Kovacs, *et al.*, *J. Am. Chem. Soc.*, **1963**, *85*, 183), an o-nitrophenyl ester (Bodanzsky, *Nature*, **1955**, *175*, 685), an imidazole ester (Li, *et al.*, *J. Am. Chem. Soc.*, **1970**, *92*, 7608), and a 3-hydroxy-4-oxo-3,4-dihydroquinazoline (Dhbt-OH) ester (Konig, *et al.*, *Chem. Ber.*, **1973**, *103*, 2024

and 2034), or the initial formation of an anhydride such as a symmetrical anhydride (Wieland, *et al.*, *Angew. Chem., Int. Ed. Engl.*, **1971**, *10*, 336). Alternatively, the carboxyl group of the incoming amino acid can be reacted directly with the N-terminal of the last-coupled amino acid with the assistance of a condensation reagent such as, for example, dicyclohexylcarbodiimide (Sheehan, *et al.*, *J. Am. Chem. Soc.*, **1955**, 77, 1067) or derivatives thereof. Benzotriazolyl N-oxytrisdimethylaminophosphonium hexafluorophosphate (BOP), "Castro's reagent" (*see, e.g.*, Rivaille, *et al.*, *Tetrahedron*, **1980**, *36*, 3413) is recommended when assembling PNA molecules containing secondary amino groups. Finally, activated PNA monomers analogous to the recently-reported amino acid fluorides (Carpino, *J. Am. Chem. Soc.*, **1990**, *112*, 9651) hold considerable promise to be used in PNA synthesis as well.

Following assembly of the desired PNA chain, including protecting groups, the next step will normally be deprotection of the amino acid moieties of the PNA chain and cleavage of the synthesized PNA from the solid support. These processes can take place substantially simultaneously, thereby providing the free PNA molecule in the desired form. Alternatively, in cases in which condensation of two separately synthesized PNA chains is to be carried out, it is possible by choosing a suitable spacer group at the start of the synthesis to cleave the desired PNA chains from their respective solid supports (both peptide chains still incorporating their side-chain protecting groups) and finally removing the side-chain protecting groups after, for example, coupling the two side-chain protected peptide chains to form a longer PNA chain.

In the above-mentioned "Boc-benzyl" protection scheme, the final deprotection of side-chains and release of the PNA molecule from the solid support is most often carried out by the use of strong acids such as anhydrous HF (Sakakibara, *et al.*, *Bull. Chem. Soc. Jpn.*, **1965**, *38*, 4921), boron tris (trifluoroacetate) (Pless, *et al.*, *Helv. Chim. Acta*, **1973**, *46*, 1609), and sulfonic acids such as

trifluoromethanesulfonic acid and methanesulfonic acid (Yajima, *et al.*, *J. Chem. Soc., Chem. Comm.*, **1974**, 107). This conventional strong acid (*e.g.*, anhydrous HF) deprotection method, produces very reactive carbocations that may lead to alkylation and acylation of sensitive residues in the PNA chain. Such side-reactions are only partly avoided by the presence of scavengers such as anisole, phenol, dimethyl sulfide, and mercaptoethanol and, therefore, the sulfide-assisted acidolytic S_N2 deprotection method (Tam, *et al.*, *J. Am. Chem. Soc.*, **1983**, *105*, 6442 and *J. Am. Chem. Soc.*, **1986**, *108*, 5242), the so-called "low", which removes the precursors of harmful carbocations to form inert sulfonium salts, is frequently employed in peptide and PNA synthesis, either solely or in combination with "high" methods. Less frequently, in special cases, other methods used for deprotection and/or final cleavage of the PNA-solid support bond are, for example, such methods as base-catalyzed alcoholysis (Barton, *et al.*, *J. Am. Chem. Soc.*, **1973**, *95*, 4501), and ammonolysis as well as hydrazinolysis (Bodanszky, *et al.*, *Chem. Ind.*, **1964** 1423), hydrogenolysis (Jones, *Tetrahedron Lett.* **1977** 2853 and Schlatter, *et al.*, *Tetrahedron Lett.* **1977** 2861)), and photolysis (Rich and Gurwara, *J. Am. Chem. Soc.*, **1975** *97*, 1575)).

Finally, in contrast with the chemical synthesis of "normal" peptides, stepwise chain building of achiral PNAs such as those based on aminoethylglycyl backbone units can start either from the N-terminus or the C-terminus, because the coupling reactions are free of racemization. Those skilled in the art will recognize that whereas syntheses commencing at the C-terminus typically employ protected amine groups and free or activated acid groups, syntheses commencing at the N-terminus typically employ protected acid groups and free or activated amine groups.

Based on the recognition that most operations are identical in the synthetic cycles of solid-phase peptide synthesis (as is also the case for solid-phase PNA synthesis), a new matrix, PEPS, was recently introduced

(Berg, *et al.*, *J. Am. Chem. Soc.*, **1989**, *111*, 8024 and International Patent Application WO 90/02749) to facilitate the preparation of large numbers of peptides. This matrix is comprised of a polyethylene (PE) film with pendant long-chain polystyrene (PS) grafts (molecular weight on the order of 10^6). The loading capacity of the film is as high as that of a beaded matrix, but PEPS has the additional flexibility to suit multiple syntheses simultaneously. Thus, in a new configuration for solid-phase peptide synthesis, the PEPS film is fashioned in the form of discrete, labeled sheets, each serving as an individual compartment. During all the identical steps of the synthetic cycles, the sheets are kept together in a single reaction vessel to permit concurrent preparation of a multitude of peptides at a rate close to that of a single peptide by conventional methods. It was reasoned that the PEPS film support, comprising linker or spacer groups adapted to the particular chemistry in question, should be particularly valuable in the synthesis of multiple PNA molecules, these being conceptually simple to synthesize since only four different reaction compartments are normally required, one for each of the four "pseudo-nucleotide" units. Thus, the PEPS film support has been successfully tested in a number of PNA syntheses carried out in a parallel and substantially simultaneous fashion. The yield and quality of the products obtained from PEPS were comparable to those obtained by using the traditional polystyrene beaded support. Also, experiments with other geometries of the PEPS polymer such as, for example, non-woven felt, knitted net, sticks or microwellplates have not indicated any limitations of the synthetic efficacy.

Two other methods proposed for the simultaneous synthesis of large numbers of peptides also apply to the preparation of multiple, different PNA molecules. The first of these methods (Geysen, *et al.*, *Proc. Natl. Acad. Sci. USA*, **1984**, *81*, 3998) utilizes acrylic acid-grafted polyethylene-rods and 96-microtiter wells to immobilize the growing peptide chains and to perform the compartmentalized

synthesis. While highly effective, the method is only applicable on a microgram scale. The second method (Houghten, *Proc. Natl. Acad. Sci. USA*, **1985**, *82*, 5131) utilizes a "tea bag" containing traditionally-used polymer beads. Other relevant proposals for multiple peptide or PNA synthesis in the context of the present invention include the simultaneous use of two different supports with different densities (Tregear, in "*Chemistry and Biology of Peptides*", J. Meienhofer, ed., Ann Arbor Sci. Publ., Ann Arbor, **1972** pp. 175-178), combining of reaction vessels via a manifold (Gorman, *Anal. Biochem.*, **1984**, *136*, 397), multicolumn solid-phase synthesis (e.g. Krchnak, *et al.*, *Int. J. Peptide Protein Res.*, **1989**, *33*, 209), and Holm and Meldal, in "*Proceedings of the 20th European Peptide Symposium*", G. Jung and E. Bayer, eds., Walter de Gruyter & Co., Berlin, **1989** pp. 208-210), and the use of cellulose paper (Eichler, *et al.*, *Collect. Czech. Chem. Commun.*, **1989**, *54*, 1746).

While the conventional cross-linked styrene/divinylbenzene copolymer matrix and the PEPS support are presently preferred in the context of solid-phase PNA synthesis, a non-limiting list of examples of solid supports which may be of relevance are: (1) Particles based upon copolymers of dimethylacrylamide cross-linked with N,N'-bisacryloylethylenediamine, including a known amount of N-tertbutoxycarbonyl-beta-alanyl-N'-acryloylhexamethylenediamine. Several spacer molecules are typically added via the beta alanyl group, followed thereafter by the amino acid residue subunits. Also, the beta alanyl-containing monomer can be replaced with an acryloyl sarcosine monomer during polymerization to form resin beads. The polymerization is followed by reaction of the beads with ethylenediamine to form resin particles that contain primary amines as the covalently linked functionality. The polyacrylamide-based supports are relatively more hydrophilic than are the polystyrene-based supports and are usually used with polar aprotic solvents including dimethylformamide, dimethylacetamide, N-methylpyrrolidone and the

like (see Atherton, *et al.*, *J. Am. Chem. Soc.*, **1975**, *97*, 6584, *Bioorg. Chem.* **1979**, *8*, 351), and J.C.S. Perkin I 538 (1981)); (2) a second group of solid supports is based on silica-containing particles such as porous glass beads and silica gel. One example is the reaction product of trichloro-[3-(4-chloromethyl)phenyl]propylsilane and porous glass beads (see Parr and Grohmann, *Angew. Chem. Internal. Ed.* **1972**, *11*, 314) sold under the trademark "PORASIL E" by Waters Associates, Framingham, MA, USA. Similarly, a mono ester of 1,4-dihydroxymethylbenzene and silica (sold under the trademark "BIOPAK" by Waters Associates) has been reported to be useful (see Bayer and Jung, *Tetrahedron Lett.*, **1970**, 4503); (3) a third general type of useful solid supports can be termed composites in that they contain two major ingredients: a resin and another material that is also substantially inert to the organic synthesis reaction conditions employed. One exemplary composite (see Scott, *et al.*, *J. Chrom. Sci.*, **1971**, *9*, 577) utilized glass particles coated with a hydrophobic, cross-linked styrene polymer containing reactive chloromethyl groups, and was supplied by Northgate Laboratories, Inc., of Hamden, CT, USA. Another exemplary composite contains a core of fluorinated ethylene polymer onto which has been grafted polystyrene (see Kent and Merrifield, *Israel J. Chem.* **1978**, *17*, 243) and van Rietschoten in "*Peptides 1974*", Y. Wolman, Ed., Wiley and Sons, New York, **1975**, pp. 113-116); and (4) contiguous solid supports other than PEPS, such as cotton sheets (Lebl and Eichler, *Peptide Res.* **1989**, *2*, 232) and hydroxypropylacrylate-coated polypropylene membranes (Daniels, *et al.*, *Tetrahedron Lett.* **1989**, 4345), are suited for PNA synthesis as well.

Whether manually or automatically operated, solid-phase PNA synthesis in the context of the present invention is normally performed batchwise. However, most of the syntheses may equally well be carried out in the continuous-flow mode, where the support is packed into columns (Bayer, *et al.*, *Tetrahedron Lett.*, **1970**, 4503 and Scott, *et al.*, *J.*

Chromatogr. Sci., **1971**, *9*, 577). With respect to continuous-flow solid-phase synthesis, the rigid poly(dimethylacrylamide)-Kieselguhr support (Atherton, *et al.*, *J. Chem. Soc. Chem. Commun.*, **1981**, 1151) appears to be particularly successful, but another valuable configuration concerns the one worked out for the standard copoly(styrene-1%-divinylbenzene) support (Krchnak, *et al.*, *Tetrahedron Lett.*, **1987**, 4469).

While the solid-phase technique is presently preferred in the context of PNA synthesis, other methodologies or combinations thereof, for example, in combination with the solid-phase technique, apply as well: (1) the classical solution-phase methods for peptide synthesis (*e.g.*, Bodanszky, "*Principles of Peptide Synthesis*", Springer-Verlag, Berlin-New York **1984**), either by stepwise assembly or by segment/fragment condensation, are of particular relevance when considering especially large scale productions (gram, kilogram, and even tons) of PNA compounds; (2) the so-called "liquid-phase" strategy, which utilizes soluble polymeric supports such as linear polystyrene (Shemyakin, *et al.*, *Tetrahedron Lett.*, **1965**, 2323) and polyethylene glycol (PEG) (Mutter and Bayer, *Angew. Chem., Int. Ed. Engl.*, **1974**, *13*, 88), is useful; (3) random polymerization (*see, e.g.*, Odian, "*Principles of Polymerization*", McGraw-Hill, New York (1970)) yielding mixtures of many molecular weights ("polydisperse") peptide or PNA molecules are particularly relevant for purposes such as screening for antiviral effects; (4) a technique based on the use of polymer-supported amino acid active esters (Fridkin, *et al.*, *J. Am. Chem. Soc.*, **1965**, *87*, 4646), sometimes referred to as "inverse Merrifield synthesis" or "polymeric reagent synthesis", offers the advantage of isolation and purification of intermediate products, and may thus provide a particularly suitable method for the synthesis of medium-sized, optionally protected, PNA molecules, that can subsequently be used for fragment condensation into larger PNA molecules; (5) it is envisaged that PNA molecules may be assembled enzymatically by enzymes such as proteases

or derivatives thereof with novel specificities (obtained, for example, by artificial means such as protein engineering). Also, one can envision the development of "PNA ligases" for the condensation of a number of PNA fragments into very large PNA molecules; (6) since antibodies can be generated to virtually any molecule of interest, the recently developed catalytic antibodies (abzymes), discovered simultaneously by the groups of Lerner (Tramantano, *et al.*, *Science*, **1986**, *234*, 1566) and of Schultz (Pollack, *et al.*, *Science*, **1986**, *234*, 1570), should also be considered as potential candidates for assembling PNA molecules. Thus, there has been considerable success in producing abzymes catalyzing acyl-transfer reactions (see for example Shokat, *et al.*, *Nature*, **1989**, *338*, 269) and references therein). Finally, completely artificial enzymes, very recently pioneered by Stewart's group (Hahn, *et al.*, *Science*, **1990**, *248*, 1544), may be developed to suit PNA synthesis. The design of generally applicable enzymes, ligases, and catalytic antibodies, capable of mediating specific coupling reactions, should be more readily achieved for PNA synthesis than for "normal" peptide synthesis since PNA molecules will often be comprised of only four different amino acids (one for each of the four native nucleobases) as compared to the twenty natural by occurring (proteinogenic) amino acids constituting peptides. In conclusion, no single strategy may be wholly suitable for the synthesis of a specific PNA molecule, and therefore, sometimes a combination of methods may work best.

Peptide nucleic acid oligomers hybridizable with, or targeted to, viral targets are provided by the present invention. By hybridizable is meant that at least 70% sequence homology is present. In preferred embodiments of the present invention, peptide nucleic acid oligomers have at least 85% sequence homology to a desired target. In still more preferred embodiments of the present invention, peptide nucleic acid oligomers of the present invention are at least 95% homologous to a target of interest.

Oligomers of the present invention comprising PNA subunits can be used in diagnostics, therapeutics and as research reagents and kits. Diagnostic and research reagents may be employed by contacting a cell or other biological sample such as blood, urine, cerebral fluid, ascites, etc. with oligomers of the present invention *in vitro*.

Oligomers of the invention can be formulated in a pharmaceutical composition, which can include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the oligomer. Pharmaceutical compositions also can include one or more active ingredients such as antimicrobial agents, anti-inflammatory agents, anesthetics, and the like in addition to oligomer.

The pharmaceutical composition can be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administration can be topically (including opthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous drip, subcutaneous, intraperitoneal or intramuscular injection.

Formulations for topical administration can include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms may also be useful.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable.

Formulations for parenteral administration can include sterile aqueous solutions which also can contain buffers, diluents and other suitable additives.

Dosing is dependent on severity and responsiveness of the condition to be treated, but will normally be one or

more doses per day, with course of treatment lasting from several days to several months or until a cure is effected or a diminution of disease state is achieved. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates.

Such methodologies will be useful for targeting the following targets for treatment of viral manifestations.

Cytomegalovirus (CMV)

In permissive human fibroblasts, CMV gene expression is regulated by a cascade of genetic events that act at both the transcriptional and translational levels. CMV gene expression can be divided into three phases which resemble those of HSV defined as the immediate early (IE), early and late periods. Following adsorption, penetration and uncoating of the virus, a group of viral transcripts, immediate early messenger RNAs (IE mRNAs) are synthesized within 1-4 hours even in the presence of translational inhibitors such as cycloheximide. In the normal course of infection, the IE mRNAs are translated and their protein products are instrumental in the onset of early transcriptional events. At least 4 proteins are synthesized from IE mRNAs; of these, one is a glycoprotein. The IE1 and IE2 proteins are transcriptional activating factors for other CMV genes and the IE3 protein encompasses a region of the CMV genome which can transform NIH 3T3 cells *in vitro*. Early proteins are encoded by the mRNAs which are synthesized prior to viral DNA synthesis. A number of the early proteins play a role in nucleotide metabolism and DNA synthesis in the infected cell. After the onset of viral DNA synthesis, the transcription of the late mRNAs is maximal and probably reflects a template abundancy requirement similar to that observed for analogous HSV mRNAs. The late CMV proteins include the glycoprotein constituents of the viral envelope, the viral capsid proteins and other proteins which are necessary for assembly or structural integrity of the mature CMV particle and/or egress of the assembled virion from the

infected cell. In addition to the transcriptional controls operant upon CMV gene expression, examples of post-transcriptional controls are known to influence the appearance of some CMV proteins. Splicing of mRNAs is more common than observed in HSV gene expression and the nucleotide sequence composition of the 5′ nontranslated region in the cognate mRNA is reported to influence the synthesis of at least one early CMV protein.

The selected targets within the mRNA sequences include regions of the mRNA which are known to control mRNA stability, processing and/or translational efficiency. These target sites include the 5′ cap regions and translation initiation control regions.

A series of antiviral oligomers comprising PNA targeted to the translation initiation codon (AUG), coding sequence, 5′ CAP, intron/exon (I/E) junction or 5′ untranslated region (5′ UTR) of CMV gene selected from the group consisting of DNA polymerase (DNA pol), and nuclear localization signals (nuc sig) of IE1 and IE2 are developed with specific oligomer sequences. The oligomer sequences, SEQ ID numbers and targets of these oligomer are shown in Table 1.

TABLE 1

SEQUENCE	TARGET	SEQ ID NO:
GGGTTGAAAAACATAGCGGAC	DNA pol AUG	1
GAGGACTCCATCGTGTCAAG	IE1 AUG	2
GTGGGCCATGATGATGGAAGG	DNA pol 5′ UTR	3
GTCCCGTAGATGACCCGCGCC	DNA pol 5′ UTR	4
CGGCGCAGATTGCAAGGGCGG	DNA pol 5′ UTR	5
GCCGGAGCCGGGTGAAACGCC	DNA pol 5′ UTR	6
CGCCGTCCGGACACCGGGCGC	DNA pol 5′ UTR	7
ACCGGGAAACCACGCCGGCGG	DNA pol 5′ UTR	8
CCGCGCCCTCTTCTTTGCCGG	DNA pol 5′ UTR	9
GGTACTTACGTCACTCTTGGC	IE1 I/E-1	10
GACGGTGACTGCAGAAAAGAC	IE1 I/E-2	11
GACACGTACCGTGGCACCTTG	IE1 I/E-3	12

GTCTCGGGCCTAAACACATGG	IE1 I/E-4	13
CAGACTTACCGACTTCTGCCG	IE1 I/E-5	14
CTGTTTGACTGTAGAGGAGGG	IE1 I/E-6	15
GGGTCCTTCATCTGGGAGAGC	IE2 AUG	16
CGGCTCACCTCGTCAATCTTG	IE2 I/E-1	17
GCGCACCATGACCTGTTTGGG	IE2 I/E-2	18
CGTCTCCAGGCGATCTGACGC	IE1/IE2 5' CAP	19
TGGCGTCTCCAGGCGATCTGA	IE1/IE2 5' CAP	20
TGGCGTCTCCAGGCGATCTGA-K		21
GTTTTGCGCGGTTTCTTACGC	IE2 nuc sig 1	22
GCGTTTGCTCTTCTTCTTGCG	IE2 nuc sig 2	23
GTTTGCTCTTCTTCTTG	IE2 nuc sig 2	24
CGTTTGCTCTTCTTCTTGC	IE2 nuc sig 2	25
GCGTTTTCTCTTCTGCTTGCG	IE2 nuc sig 2	26
TCGGTTTCTCGTCTGCTTTCG	IE2 nuc sig 2	27
GCGGTTTCTCTTCTGCTTTCG	IE2 nuc sig 2	28
TATGGAGGTCAAAACAGCGTG	IE 5' UTR	29
TGGATCGGTCCCGGTGTCTTC	IE 5' UTR	30
ACCGTTCCCGGCCGCGGAGGC	IE 5' UTR	31
GGGGAATCCGCGTTCCAATGC	IE 5' UTR	32
CACCCGCGACCGCACCGCCGG	DNA pol coding	33
CAGATACGGGTTGAAAAACAT	DNA pol AUG	34
TGGTGTAAGGCGGAGCCGCCG	DNA pol 5' UTR	35
TGGTGTAAGGCGGGGCCGCCG	DNA pol 5' UTR	36
CAGACGGGCCAGGGCCAGAAG	DNA pol 5' UTR	37
CAGACGGGCCGGGGCCAGAAG	DNA pol 5' UTR	38
TCCTGCGTGCCAGTCTGTCCG	DNA pol 5' UTR	39
GTAGCCGTTTTTGCGATGTCG	DNA pol 5' UTR	40
CCTCCTGGTTCAGACGTTCTC	DNA pol 5' UTR	41
CAGTTTAACCCCGTATATCAC	DNA pol 5' UTR	42
CAGCTTACGAAGCAAAATCAC	DNA pol 5' UTR	43
CATAGCGGACCGTGAGAGGCT	DNA pol AUG	44
CATAGCGGACCGTGGGAGGCT	DNA pol AUG	45
CATAGCGGACCGTGAGGGGCT	DNA pol AUG	46
CATAGCGGACCGTGGGGGGCT	DNA pol AUG	47
AAACCCACGGCGGGGCTGTGT	DNA pol coding	48
CGCGCGATGGCCCCGGCCTGC	DNA pol coding	49

GCGTTTGCTCTTCTTCTTGC	IE2 nuc sig 2	50
CGTTTGCTCTTCTTCTTGCG	IE2 nuc sig 2	51
GCGTTTGCTCTTCTTCTTG	IE2 nuc sig 2	52
GCGTTTGCTCTTCTTCTT	IE2 nuc sig 2	53
GCGTTTGCTCTTCTTCT	IE2 nuc sig 2	54
GTTTGCTCTTCTTCTTGCG	IE2 nuc sig 2	55
TTTGCTCTTCTTCTTGCG	IE2 nuc sig 2	56
TTGCTCTTCTTCTTGCG	IE2 nuc sig 2	57
GGACCGGGACCACCGTCGTC	DNA pol 5' CAP	58
GTCCGCTATGTTTTTCAACCC	DNA pol AUG	59
CCTTCCATCATCATGGCCCAC	DNA pol coding	60
GGCGCGGGTCATCTACGGGAC	DNA pol coding	61
CCGCTGTGCCCGGCGACGCGG CCGCCCTTGC	DNA pol 5' UTR	62
AATCTGCGCCGGGCGTTTCAC CCGGCTCCGGC	DNA pol 5' UTR	63
GCGCCCGGTGTCCGGACGGCG CCGCCGGCGT	DNA pol 5' UTR	64
GGTTTCCCGGTCCGGCAAAGA AGAGGGCGCGG	DNA pol 5' UTR	65
GTGAACCGTCAGATCGCCTGG	IE1 5' CAP	66
CTTGACACGATGGAGTCCTC	IE1 AUG	67
GCCAAGAGTGACGTAAGTACC	IE1 I/E-1	68
GTCTTTTCTGCAGTCACCGTC	IE1 I/E-2	69
CAAGGTGCCACGGTACGTGTC	IE1 I/E-3	70
CATGTGTTTAGGCCCGAGAC	IE1 I/E-4	71
GGCAGAACTCGGTAAGTCTG	IE1 I/E-5	72
CCTCCTCTACAGTCAAACAG	IE1 I/E-6	73
GCGCCTATCATGCTGCCCCTC	IE2 AUG	74
GCTCTCCCAGATGAACCACCC	IE2 AUG	75
CAAGATTGACGAGGTGAGCCG	IE2 I/E-1	76
CCCAAACAGGTCATGGTGCGC	IE2 I/E-2	77
GCGTAAGAAACCGCGCAAAAC	IE2 nuc sig 1	78
CGCAAGAAGAAGAGCAAACGC	IE2 nuc sig 2	79

Papillomaviruses

Papillomaviruses, especially HPV-1, HPV-2, HPV-3, HPV-4, HPV-6, HPV-10, HPV-11, HPV-16, HPV-18, HPV-31, and HPV-33, HPV-35 are of particular interest in some embodiments

of the present invention. It is preferred that portions of the papillomavirus genome including mRNA derived from open reading frames E1, E2, E4, E5, E6, E7 (early open reading frames), L1 and L2 (late open reading frames) are targeted by PNA oligomers of the present invention. The oligomer sequences, SEQ ID numbers and region targeted within the papilloma genomes are shown in Table 2

Table 2

SEQUENCE	TARGET	SEQ ID NO:
AGGTTTGCACCCGAC TATGCAAGTACAAAT	mRNA cap region	80
TATGCAAGTACAAAT	mRNA cap region	81
CGTTCGCATGCTGTC TCCATCCTCTTCACT	initiation of trans	82
GCATGCTGTCTCCAT	initiation of trans	83
AAATGCGTCCAGCAC CGGCCATGGTGCAGT	transrepressor start	84
AGCACCGGCCATGGT	transrepressor start	85
CAATGGCAGTGATCA GAAGTCCAAGCTGGC	translational termin	86
GCAGTGATCAGAAGT	translational termin	87
ATTGCTGCAGCTTAA ACCATATAAAATCTG	3′ untranslated region	88
CTTAAACCATATAAA	3′ untranslated region	89
AAAAAAAGATTTCCA ATCTGCATCAGTAAT	5′ untranslated region	90
AAGATTTCCAATCTG	5′ untranslated region	91
CAGTGTCCTAGGACA GTCACCCCTTTTTTC	5′ coding region	92
GGACAGTCACCCCTT	5′ coding region	93
TGTACAAATTGCTGT AGACAGTGTACCAGT	mid coding region	94
GCTGTAGACAGTGTA	mid coding region	95

GTGCGAGCGAGGACC GTCCCGTACCCAACC	3′ coding region	96
GGACCGTCCCGTACC	3′ coding region	97
TTTAACAGGTGGAAT CCATCATTGGTGGTG	5′ coding region	98
GGAATCCATCATTGG	5′ coding region	99
GCTTCCATCTTCCTC	trans init. codon	100
GCTTCCATCTTCCTCG	trans. init. codon	101
TGCTTCCATCTTCCTCG	trans. init. codon	102
TGCTTCCATCTTCCTCGT	trans. init. codon	103
TTGCTTCCATCTTCCTCGT	trans. init. codon	104
TTGCTTCCATCTTCCTCGTC	trans. init. codon	105
CGACTATGCAAGTAC		106
CGACTATGCAATTTC		107
TTTCTATGCAAGTAC		108
CGACTATGCAACCCC		109
TCTCCATCCTCTTCACT		110
AGCGCGCCATAGTATTGTGG	E6 trans. init. codon	111
GTCCATGCATACTTAATATT	E7 trans. init. codon	112
TATTACGTACTAGATTCTAC		113
CTGTCTCCATCCTCTTCACT	E2 trans. init. codon	114.

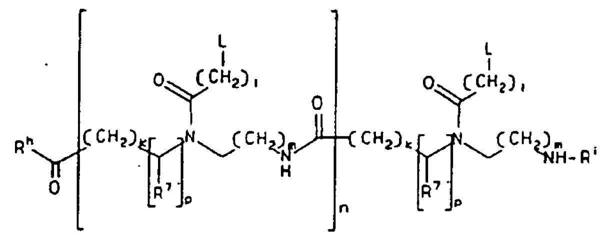
The following examples are provided for illustrative purposes only and are not intended to limit the invention.

Example 1

General Method for the Synthesis of Antiviral Oligomers Comprising PNA

PNA subunits for oligomers of the invention are prepared generally in accordance with the methods disclosed by WO 92/20702, incorporated by reference herein in its entirety. Benzyhydrylamine resin (initially loaded 0.28 mmol/gm with Boc-L-Lys(2-chlorobenyloxycarbonyl)) is swollen in DMF and an excess of a monomer to be coupled is added, followed by dicyclohexylcarbodiimide (0.15M in 50% DMF in dichloromethane). The Boc deprotection is accomplished by

trifluoroacetic acid treatment. The progress of the coupling reactions is monitored by quantitative ninhydrin analysis. The PNA is released from the resin using anhydrous HF under standard conditions. The products are purified using HPLC with acetonitrile-water (0.1%TFA) gradient and structure confirmed by fast atom bombardment mass spectrometry. PNA homopolymer has the structure



wherein k is 1; m is 1; l is 1; p is 0; R^h is OH; R^i is H; and n is the number of bases in the oligomer sequence minus 1.

Example 2

Inhibition of CMV with Antiviral Oligomers Comprising PNA

Human foreskin fibroblast (ATCC #CRL 1635) cells used are obtained from the American Tissue Culture Collection. Cultures are grown in Dulbecco's Modified Eagle's Medium with 4.5 g/L glucose (high glucose DMEM) and supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/ml), streptomycin (100 micrograms/ml) and L-glutamine (2 mM). Stock cultures of human cytomegalovirus (HCMV strain AD169 or Towne) are grown on foreskin cells using low multiplicity infections (multiplicity of infection [MOI]=0.02 plaque forming units [PFU]/cell).

Antiviral oligomers comprising PNA prepared in accordance with Example 1, having the following oligomer

sequences: GGGTTGAAAAACATAGCGGAC (SEQ ID NO: 1), GAGGACTCCATCGTGTCAAG (SEQ ID NO: 2), GTGGGCCATGATGATGGAAGG (SEQ ID NO: 3), GTCCCGTAGATGACCCGCGCC (SEQ ID NO: 4), CGGCGCAGATTGCAAGGGCGG (SEQ ID NO: 5), GCCGGAGCCGGGTGAAACGCC (SEQ ID NO: 6), CGCCGTCCGGACACCGGGCGC (SEQ ID NO: 7), ACCGGGAAACCACGCCGGCGG (SEQ ID NO: 8), CCGCGCCCTCTTCTTTGCCGG (SEQ ID NO: 9), GGTACTTACGTCACTCTTGGC (SEQ ID NO: 10), GACGGTGACTGCAGAAAAGAC (SEQ ID NO: 11), GACACGTACCGTGGCACCTTG (SEQ ID NO: 12), GTCTCGGGCCTAAACACATG (SEQ ID NO: 13), CAGACTTACCGACTTCTGCC (SEQ ID NO: 14), CTGTTTGACTGTAGAGGAGG (SEQ ID NO: 15), GGGTCCTTCATCTGGGAGAGC (SEQ ID NO: 16), CGGCTCACCTCGTCAATCTTG (SEQ ID NO: 17), GCGCACCATGACCTGTTTGGG (SEQ ID NO: 18), CGTCTCCAGGCGATCTGACGC (SEQ ID NO: 19), TGGCGTCTCCAGGCGATCTGA (SEQ ID NO: 20), GTTTTGCGCGGTTTCTTACGC (SEQ ID NO: 22), GCGTTTGCTCTTCTTCTTGCG (SEQ ID NO: 23), GTTTGCTCTTCTTCTTG (SEQ ID NO: 24), CGTTTGCTCTTCTTCTTGC (SEQ ID NO: 25), GCGTTTTCTCTTCTGCTTGCG (SEQ ID NO: 26), TCGGTTTCTCGTCTGCTTTCG (SEQ ID NO: 27), GCGGTTTCTCTTCTGCTTTCG (SEQ ID NO: 28), TATGGAGGTCAAAACAGCGTG (SEQ ID NO: 29), TGGATCGGTCCCGGTGTCTTC (SEQ ID NO: 30), ACCGTTCCCGGCCGCGGAGGC (SEQ ID NO: 31), GGGGAATCCGCGTTCCAATGC (SEQ ID NO: 32), CACCCGCGACCGCACCGCCGG (SEQ ID NO: 33), CAGATACGGGTTGAAAAACAT (SEQ ID NO: 34), TGGTGTAAGGCGGAGCCGCCG (SEQ ID NO: 35), TGGTGTAAGGCGGGGCCGCCG (SEQ ID NO: 36), CAGACGGGCCAGGGCCAGAAG (SEQ ID NO: 37), CAGACGGGCCGGGGCCAGAAG (SEQ ID NO: 38), TCCTGCGTGCCAGTCTGTCCG (SEQ ID NO: 39), GTAGCCGTTTTTGCGATGTCG (SEQ ID NO: 40), CCTCCTGGTTCAGACGTTCTC (SEQ ID NO: 41), CAGTTTAACCCCGTATATCAC (SEQ ID NO: 42), CAGCTTACGAAGCAAAATCAC (SEQ ID NO: 43), CATAGCGGACCGTGAGAGGCT (SEQ ID NO: 44), CATAGCGGACCGTGGGAGGCT (SEQ ID NO: 45), CATAGCGGACCGTGAGGGGCT (SEQ ID NO: 46), CATAGCGGACCGTGGGGGGCT (SEQ ID NO: 47), AAACCCACGGCGGGGCTGTGT (SEQ ID NO: 48), CGCGCGATGGCCCCGGCCTGC (SEQ ID NO: 49), GCGTTTGCTCTTCTTCTTGC (SEQ ID NO: 50), CGTTTGCTCTTCTTCTTGCG (SEQ ID NO: 51), GCGTTTGCTCTTCTTCTTG (SEQ ID NO: 52), GCGTTTGCTCTTCTTCTT (SEQ ID NO: 53), GCGTTTGCTCTTCTTCT (SEQ ID NO: 54), GTTTGCTCTTCTTCTTGCG (SEQ ID NO: 55), TTTGCTCTTCTTCTTGCG (SEQ ID NO: 56) and

TTGCTCTTCTTCTTGCG (SEQ ID NO: 57), are employed in this assay.

To assess the ability of oligomers to inhibit CMV replication, an infectious yield assay will be used. To perform this assay, foreskin cells are seeded at a density of 5 X 10^5 cells per well in Falcon 6 well tissue culture plates. Cells are overlaid with 2 ml of medium (high glucose DMEM with 10% FBS) and incubated at 37°C for 18-24 hours. Where appropriate, cells are overlaid with oligomer preparations in 1 ml of medium at 24 hours after seeding the plates. Following an 18 hour incubation, all wells are rinsed with PBS and infected with HCMV at varying MOIs suspended in 0.5 ml of serum-free hgh glucose DMEM. Virus and cells are incubated at 37°C for 90 minutes on a rocking platform. Following viral adsorption, unadsorbed virus is rinsed away by washing with PBS. Where appropriate, 1 ml of medium (high glucose DMEM with 10% FBS) containing 10 μM concentrations of oligomer are added to the well and the cells are incubated for 4-5 days at 37°C. Control wells receive 1 ml of medium which contains no oligomer.

Virus is harvested into the overlay medium and triplicate wells of each experimental point are combined. The suspension is frozen at -80°C. Virus titer is determined for each sample by plaque assay on human foreskin cell monolayers. Dilutions of each virus preparation are prepared and duplicate aliquots of each dilution are absorbed onto foreskin cells for 90 minutes with rocking. After adsorption, the unadsorbed virus inoculum is removed by rinsing the plates with PBS and the cells are overlaid with 2 ml of high glucose DMEM containing 5% FBS and 0.75% methyl cellulose. Cells are incubated at 37°C for 12-14 days before plaques are fixed with formalin, stained with crystal violet and counted. Plaque counts from treated wells are compared with those from the control wells to establish the degree of inhibition of infectious virus production. It is expected that treatment of cells with oligomers directed to CMV will reduce the infectious yield of same cells.

Example 3

ELISA Assay for Inhibition of HCMV Replication by Antiviral Oligomers Comprising PNA

The aforementioned oligomers, complementary to human cytomegalovirus mRNA, are tested for antiviral activity in an ELISA-based assay of HCMV replication. Normal human dermal fibroblasts (Clonetics Corp., San Diego CA) are grown in serum-free medium (Clonetics) and used to seed 96-well plates. When cells are approximately 80% confluent, they are pretreated with oligomers. Approximately 20 hours after pretreatment the medium (containing oligomer) is carefully removed and the cells washed twice with warmed fibroblast basal medium (FBM, Clonetics). Cells are then infected with 100 µl/well of CMV stock diluted in FBM. The plates are incubated at 37°C for two hours. The medium (containing virus) is then carefully removed and replaced with fresh, prewarmed FBM medium, 100 µl per well. The plates are briefly incubated at 37°C and then 5 µl of oligomer, diluted in FBM, is reintroduced into the medium in each well. Two days later, cells are post-treated again with oligomer in the same way. On day six, the plates are prepared for ELISA.

In preparation for ELISA, the medium is carefully removed from the plates, and cells are fixed in 200 µl of absolute ethanol per well. Cells are fixed for 30 minutes at room temperature, then ethanol is removed and plates are air-dried. Plates are blocked for one hour prior to ELISA with PBS containing 2% BSA. Blocking solution is removed and 100 µl of an anti-CMV antibody, diluted 1:2000 in PBS with 1% BSA, is added. Cells are incubated in antibody for one hour at 37°C and washed three times in PBS. The secondary antibody, biotinylated goat anti-mouse IgG (Bethesda Research Labs, MD), is diluted 1:1000 in PBS with 1% BSA, and incubated with cells for one hour at 37°C. Cells are then washed and incubated for one hour at 37°C in streptavidin-B-D-galactosidase. Color is developed with chlorophenol red-B-D-galactopyranoside, 20 mg dissolved in 10 ml of 50 mM Na phosphate, 1.5 mM $MgCl_2$; plates are shaken for 10 minutes and the absorbance is read at 575 nm. It is expected that

treatment of cells with oligomers directed to CMV will reduce the replication of HCMV resulting in less color produced upon examination by ELISA.

Example 4

Inhibition of Expression of BPV-1 E2 by Antisense Oligomers

BPV-1 transformed C127 cells are plated in 12 well plates. Twenty four hours prior to transfection with E2RE1 cells are pretreated by addition of PNA oligomers (prepared as described in Example 1) AGGTTTGCACCCGACTATGCAAGTACAAAT (SEQ ID NO: 80), TATGCAAGTACAAAT (SEQ ID NO: 81), CGTTCGCATGCTGTCTCCATCCTCTTCACT (SEQ ID NO: 82), GCATGCTGTCTCCAT (SEQ ID NO: 83), AAATGCGTCCAGCAC CGGCCATGGTGCAGT (SEQ ID NO: 84), AGCACCGGCCATGGT (SEQ ID NO: 85), CAATGGCAGTGATCAGAAGTCCAAGCTGGC (SEQ ID NO: 86), GCAGTGATCAGAAGT (SEQ ID NO: 87), ATTGCTGCAGCTTAA ACCATATAAAATCTG (SEQ ID NO: 88), CTTAAACCATATAAA (SEQ ID NO: 89), AAAAAAAGATTTCCAATCTGCATCAGTAAT (SEQ ID NO: 90), AAGATTTCCAATCTG (SEQ ID NO: 91), CAGTGTCCTAGGACAGTCACCCCTTTTTTC (SEQ ID NO: 92), GGACAGTCACCCCTT (SEQ ID NO: 93), TGTACAAATTGCTGT AGACAGTGTACCAGT (SEQ ID NO: 94), GCTGTAGACAGTGTA (SEQ ID NO: 95), GTGCGAGCGAGGACCGTCCCGTACCCAACC (SEQ ID NO: 96), GGACCGTCCCGTACC (SEQ ID NO: 97), TTTAACAGGTGGAAT CCATCATTGGTGGTG (SEQ ID NO: 98), and GGAATCCATCATTGG (SEQ ID NO: 99) to the growth medium at final concentrations of 5, 15 and 30 mM. The next day cells are transfected with 10 μg of E2RE1CAT by calcium phosphate precipitation. Ten micrograms of E2RE1CAT and 10 μg of carrier DNA (PUC 19) are mixed with 62 μl of 2 M $CaCl_2$ in a final volume of 250 μl of H_2O, followed by addition of 250 μl of 2X HBSP (1.5 mM Na_2PO_2. 10 mM KCl, 280 mM NaCl, 12 mM glucose and 50 mM HEPES, pH 7.0) and incubated at room temperature for 30 minutes. One hundred microliters of this solution is added to each test well and allowed to incubate for 4 hours at 37°C. After incubation cells are glycerol shocked for 1 minute at room temperature with 15% glycerol in 0.75 mM Na_2PO_2, 5 mM KCl,

140 mM NaCl, 6 mM glucose and 25 mM HEPES, pH 7.0. After shocking, cells are washed 2 times with serum free DMEM and refed with DMEM containing 10% fetal bovine serum and PNA oligomers at the original concentration. Forty eight hours after transfection cells are harvested and assayed for CAT activity.

For determination of CAT activity, cells are washed 2 times with phosphate buffered saline and collected by scraping. Cells are resuspended in 100 ul of 250 mM Tris-HCl, pH 8.0 and disrupted by freeze-thawing 3 times. Twenty four microliters of cell extract is used for each assay. For each assay, the following are mixed together in an 1.5 ml Eppendorff tube: 25 μl of cell extract, 5 μl of 4 mM acetyl coenzyme A, 18 μl H_2O and 1 ul ^{14}C-chloramphenicol, 40-60 mCi/mM and incubated at 37°C for 1 hour. After incubation chloramphenicol (acetylated and nonacetylated forms) are extracted with ethyl acetate and evaporated to dryness. Samples are resuspended in 25 μl of ethyl acetate and spotted onto a TLC plate and chromatograph in chloroform:methanol (19:1). TLC are analyzed by autoradiography. Spots corresponding to acetylated and nonacetylated ^{14}C-chloramphenicol are excised from the TLC plate and counted by liquid scintillation for quantitation of CAT activity. PNA oligomers that depress CAT activity in a dose dependent fashion are considered positives.

Example 5

Inhibition of HPV E2 Expression by PNA Oligomers

The assay for inhibition of HPV E2 by PNA oligomers is essentially the same as that for BPV-1 E2. For HPV assays appropriate HPVs are co-transfected into either CV-1 or A431 cells with PSV2NEO cells using the calcium phosphate method described above. Cells which take up DNA are selected for by culturing in media containing the antibiotic G418. G418 resistant cells are then analyzed for HPV DNA and RNA. Cells expressing E2 are used as target cells for studies. For each PNA oligomer cells are pretreated as above followed by

transfection with E2RE1CAT and analysis of CAT activity as above. PNA oligomers are considered to have a positive effect if they can depress CAT activity in a dose dependent fashion.

Example 6

Inhibition of HPV E7 Expression by PNA Oligomers

The E7 of HPV-16 has been shown to be capable of transactivating the Ad E2 promoter (Phelps, W. C. Yee, C. L., Munger, K., and Howley, P. M. 1988, The Human Papillomavirus Type 16 E7 Gene Encodes Transactivation and Transformation Functions Similar to Those of Adenovirus E1A, *Cell* **53**:539-547. To monitor this activity, a plasmid is constructed which contained the chloramphenicol transferase gene under the control of the Ad E2 promoter (AdE2CAT). Under the conditions of this assay, CAT expression is dependent on expression of HPV E7. For this assay, cell lines are developed that contain the HPV E7 under the control of the SV40 early promoter. For each PNA oligomer, cells are pretreated as above followed by transfection with AdE2CAT and analysis of CAT activity as above.

Example 7

Inhibition of Expression of BPV-1 E1 by PNA Oligomers

The E1 of BPV-1 has been shown to be a regulator of viral genome replication. To test the effects of PNA oligomers on viral replication C127 cells infected with BPV-1 are treated with E1 specific PNA oligomers by addition of oligomers to the growth medium at final concentrations of 5, 15 and 30 μM. The effects of the oligomers are evaluated by a routine Northern blot analysis for quantitation of both E1 specific RNA as well as total viral RNA. In addition, the effects of PNA oligomers on viral genome copy number are determined by Southern blot on total genomic DNA.

Example 8

Determination of Efficacy of BPV-1 PNA Oligomers on Experimentally Induced Bovine Fibropapillomas

Multiple bovine fibropapillomas are induce on calves by direct infection of the epidermis with purified BPV-1. Upon development, fibropapillomas are treated with oligomers that had positive results in vitro as well as controls. Oligomers that induce regression of the fibropapilloma are considered as positives.

Example 9

Design and Synthesis of Oligomers Complementary to E2 mRNA

PNA oligomers are designed to be complementary to various regions of the E2 mRNA as defined by the published nucleotide sequence of BPV-1 (Chen, E. Y., Howley, P. M., Levinson, A. D., and Seeburg, P. H., The primary structure and genetic organization of the bovine papillomavirus type 1 genome, *Nature* **299**:529-534 (1982)) and cDNA structure of the major E2 transactivator mRNA (Yang, Y. C., Okayama, H., and Howley, P. M., Bovine papillomavirus contains multiple transforming genes, *Proc. Natl. Acad. Sci. USA* **82**:1030-1034 (1985)). PNA oligomers targeted to the translation initiation codon of HPV-11 E2 are based on the published sequence of HPV-11 (Dartmann, K., Schwarz, E., Gissamnn, L., and zur Hausen, *Virology* **151**:124-130 (1986)). PNA oligomers are prepared as described in Example 1. For use in cell culture assays, oligomers are routinely diluted to 100 micromolar stocks and stored at -80°C until use. The purity, integrity, and quantity of the oligomers preparations are determined by electrophoresis on 20% acrylamide 7 M urea gels (40 cm x 20 cm x 0.75 mm) prepared as described by Maniatis et al. (Maniatis, T., Fritsch, E. F. and Sambrook, J. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York, 1982). Electrophoresed oligomers were visualized within the gel by staining with "Stains-all" , 1-ethyl-2[3-(1-ethylnapthol[1,2-d]-thiazolin-2-ylidene)-2-Methyl-Propenyl[napthol[1,2d]-thiazolium bromide purchased

from Sigma, E-9379, (Dahlberg, A. E., Digman, C. W. and Peacock, A. C., *J. Mol. Biol.* **41**:39 (1969)).

Example 10 Molecular Constructs

The E2 chloramphenicol acetyl transferase (CAT) reporter plasmid to be used in this study is described (Spalholz, B. A., Byrne, J. C. and Howley, P. M., Evidence for Cooperativity between E2 Binding Sites in E2 trans-regulation of Bovine Papillomavirus Type 1, *J. Virol.* **62**:3143-3150 (1988)). Briefly, the E2 responsive element, E2RE1, (nt 7611-7806) of BPV-1 is reconstructed using oligomers and cloned into pSV2CAT that is deleted of the SV40 enhancer, Sph1 fragment. Expression of CAT from this plasmid has been shown to be dependent upon full length E2. Plasmid C59 will contain an E2 cDNA expressed from the simian virus 40 promoter and enhancer and has been described in detail elsewhere (Yang, Y.-C., Okayama, H. and Howley, P. M., Bovine papillomavirus contains multiple transforming genes, *Proc. Natl. Acad. Sci. USA* **82**:1030-1034 (1985)). Two HPV-11 full length E2 expression constructs are made. IPV115 will contain the XmnI fragment of HPV-11 (nt 2665-4988) cloned into the SmaI site of pMSG (Pharmacia catalog number 27-4506), IPV118 will contain the same HPV-11 XmnI fragment cloned into the SmaI site of pSVL (Pharmacia, catalog number 27-4509).

Example 11 Cell Lines

Mouse C127 cells (Dvoretzky, I. Schober, R., and Lowy, D., Focus Assay in Mouse Cells for Bovine Papillomavirus type 1, *Virology* **103**:369-375 (1980)) are grown in Dulbecco's Modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin (100U/ml), streptomycin (100ug/ml), and L-glutamine (4 mM). I-38 cell line are derived from a single focus of C127 cells transformed by purified BPV-1 (Cowsert, L. M., Lake, P., and Jenson, A. B., Topographical and conformational Epitopes of Bovine Papillomavirus type 1 Defined by Monoclonal Antibodies, *JNCI* **79**:1053-1057 (1987)).

Example 12

Oligomer Inhibition of E2 Dependent Transactivation Assays

To test an oligomer's ability to inhibit E2 transactivation or transrepression, I-38 cells are plated at 1 x 10^4 cells per cm^2 in 60 mm petri dishes 24 hours before transfection. Sixteen hours prior to transfection, media is aspirated and replaced with media containing oligomer at the appropriate concentration. One hour prior to transfection, media is aspirated and replaced with fresh media without oligomer. Cells are transfected by the calcium phosphate precipitation method as described by Graham et al. 1973 (Graham, F. L. and van der Eb, A. J., A New Technique for the Assay of Infectivity of Human Adenovirus 5 DNA, *Virology* 52:456-461 (1973)) with a total of 20 micrograms of DNA in one milliliter of precipitate. Each 60 mm dish receives 200 microliters of precipitate containing 4 mictograms of DNA. Four hours after the addition of precipitated DNA, the supernatant is aspirated and the cells are treated with 15% glycerol (Frost, E. and Williams, J., Mapping Temperature-Sensitive and host-range mutation of Adenovirus type 5 by Marker Rescue, *Virology* 91:39-50 (1978)). After washing, cells are refed with media containing oligomer at the original concentration and are incubated for 48 hours.

Example 13

PNA Oligomer Inhibition of Focus Formation

The ability of PNA oligomers that inhibited E2 transactivation to inhibit viral focus formation, a measure of transformation, is tested. Mouse C127 cells are plated at subconfluence (5 x 10^4 cells/cm^2) in 60 mm petri dishes. Cells are either infected with 50 focus forming units (FFU) per plate of purified BPV-1 or transfected with cloned BPV-1 DNA. Twenty-four hours after infection or transfection, oligomers are added to the medium. Medium is changed every 72 hours with fresh oligomer added with each change. Twenty-five days post infection, cells are fixed in 10% formalin in PBS for 5 minutes and stained with 0.14% methylene blue

aqueous solution for 10 minutes. Plates are washed with water and foci counted.

Example 14

PNA Oligomer Inhibition of Human Papillomavirus HPV-11 E2 Transactivation

The PNA oligomer having the sequence TTGCTTCCATCTTCCTCGTC (SEQ ID NO:105) is designed to hybridize to the AUG (translation initiation) region of the HPV-11 E2 transactivator mRNA. For inhibition of HPV-11 E2 transactivation, C127 cells are pretreated with oligomer by addition to the medium. The next day, medium is aspirated and replaced with fresh medium without oligomer. Cells are co-transfected with 2 μg IPV 118 HPV-11 E2 expression plasmid, 2 μg IPV120-15 D2-CAT reporter plasmid, and 2 μg PCH110. Following transfection, cells are treated again with oligomer and incubated for 48 hours. Cells are harvested and processed for CAT and ß-galactosidase assays. Chloramphenicol acetyltransferase activity is determined using standard protocols [Groman, C.M., Moffat, L.F., and Howard, B.H., *Mol. Cell. Biol.* 2:1044-1051 (1982)]. Acetylated and nonacetylated reaction products are separated by thin layer chromatography and quantitated using a Molecular Dynamics PhosphoImager (Molecular Dynamics, Sunnyvale CA). ß-galactosidase activity are determined using standard methods [Herbomel et al., *Cell* 39:653 (1984)].

Example 15

Inhibition of Human Cancer Cells by HPV-18 PNA Oligomers

HPV is implicated in both oral cancer and cervical cancer in humans. PNA oligomers corresponding to the start codon regions of the E6 and E7 genes of HPV-18; AGCGCGCCATAGTATTGTGG (SEQ ID NO:111); GTCCATGCATACTTAATATT (SEQ ID NO:112) TATTACGTACTAGATTCTAC (SEQ ID NO:113); are made in accordance with Example 1.

The HPV-18-transformed oral cancer cell line 1483 and the cervical cancer cell line C4-1 are used, both of

which contain HPV-18 DNA. Cells are plated on day 1; after cells attach, medium is aspirated and replaced with fresh medium containing 2 μM or 5 μM oligomer. Medium is aspirated and replaced with medium containing fresh oligomer on day 3. Replicate plates are harvested on days 2, 3, 4, 5 and 6, and the cells are counted.

WHAT IS CLAIMED IS:

1. An oligomer having a sequence hybridizable to AUG region, 5' untranslated region, intron/exon junction or coding sequence of cytomegalovirus gene selected from the group consisting of DNA polymerase, IE1, and IE2 and comprising at least one peptide nucleic acid subunit.

2. The oligomer of claim 1 wherein the sequence of the oligomer is selected from the group consisting of:

GGGTTGAAAAACATAGCGGAC (SEQ ID NO: 1);
GAGGACTCCATCGTGTCAAG (SEQ ID NO: 2);
GTGGGCCATGATGATGGAAGG (SEQ ID NO: 3);
GTCCCGTAGATGACCCGCGCC (SEQ ID NO: 4);
CGGCGCAGATTGCAAGGGCGG (SEQ ID NO: 5);
GCCGGAGCCGGGTGAAACGCC (SEQ ID NO: 6);
CGCCGTCCGGACACCGGGCGC (SEQ ID NO: 7);
CCGGGAAACCACGCCGGCGG (SEQ ID NO: 8);
CCGCGCCCTCTTCTTTGCCGG (SEQ ID NO: 9);
GGTACTTACGTCACTCTTGGC (SEQ ID NO: 10);
GACGGTGACTGCAGAAAAGAC (SEQ ID NO: 11);
GACACGTACCGTGGCACCTTG (SEQ ID NO: 12);
GTCTCGGGCCTAAACACATG (SEQ ID NO: 13);
CAGACTTACCGACTTCTGCC (SEQ ID NO: 14);
CTGTTTGACTGTAGAGGAGG (SEQ ID NO: 15);
GGGTCCTTCATCTGGGAGAGC (SEQ ID NO: 16);
CGGCTCACCTCGTCAATCTTG (SEQ ID NO: 17);
GCGCACCATGACCTGTTTGGG (SEQ ID NO: 18);
CGTCTCCAGGCGATCTGACGC (SEQ ID NO: 19);
TGGCGTCTCCAGGCGATCTGA (SEQ ID NO: 20);
TGGCGTCTCCAGGCGATCTGA-K (SEQ ID NO: 21);
GTTTTGCGCGGTTTCTTACGC (SEQ ID NO: 22);
GCGTTTGCTCTTCTTCTTGCG (SEQ ID NO: 23);
GTTTGCTCTTCTTCTTG (SEQ ID NO: 24);
CGTTTGCTCTTCTTCTTGC (SEQ ID NO: 25);
GCGTTTTCTCTTCTGCTTGCG (SEQ ID NO: 26);
TCGGTTTCTCGTCTGCTTTCG (SEQ ID NO: 27);

GCGGTTTCTCTTCTGCTTTCG (SEQ ID NO: 28);
TATGGAGGTCAAAACAGCGTG (SEQ ID NO: 29);
TGGATCGGTCCCGGTGTCTTC (SEQ ID NO: 30);
ACCGTTCCCGGCCGCGGAGGC (SEQ ID NO: 31);
GGGGAATCCGCGTTCCAATGC (SEQ ID NO: 32);
CACCCGCGACCGCACCGCCGG (SEQ ID NO: 33);
CAGATACGGGTTGAAAAACAT (SEQ ID NO: 34);
TGGTGTAAGGCGGAGCCGCCG (SEQ ID NO: 35);
TGGTGTAAGGCGGGGCCGCCG (SEQ ID NO: 36);
CAGACGGGCCAGGGCCAGAAG (SEQ ID NO: 37);
CAGACGGGCCGGGGCCAGAAG (SEQ ID NO: 38);
TCCTGCGTGCCAGTCTGTCCG (SEQ ID NO: 39);
GTAGCCGTTTTTGCGATGTCG (SEQ ID NO: 40);
CCTCCTGGTTCAGACGTTCTC (SEQ ID NO: 41);
CAGTTTAACCCCGTATATCAC (SEQ ID NO: 42);
CAGCTTACGAAGCAAAATCAC (SEQ ID NO: 43);
CATAGCGGACCGTGAGAGGCT (SEQ ID NO: 44);
CATAGCGGACCGTGGGAGGCT (SEQ ID NO: 45);
CATAGCGGACCGTGAGGGGCT (SEQ ID NO: 46);
CATAGCGGACCGTGGGGGGCT (SEQ ID NO: 47);
AAACCCACGGCGGGGCTGTGT (SEQ ID NO: 48);
CGCGCGATGGCCCCGGCCTGC (SEQ ID NO: 49);
GCGTTTGCTCTTCTTCTTGC (SEQ ID NO: 50);
CGTTTGCTCTTCTTCTTGCG (SEQ ID NO: 51);
GCGTTTGCTCTTCTTCTTG (SEQ ID NO: 52);
GCGTTTGCTCTTCTTCTT (SEQ ID NO: 53);
GCGTTTGCTCTTCTTCT (SEQ ID NO: 54);
GTTTGCTCTTCTTCTTGCG (SEQ ID NO: 55);
TTTGCTCTTCTTCTTGCG (SEQ ID NO: 56);
TTGCTCTTCTTCTTGCG (SEQ ID NO: 57);
GGACCGGGACCACCGTCGTC (SEQ ID NO: 58);
GTCCGCTATGTTTTTCAACCC (SEQ ID NO: 59);
CCTTCCATCATCATGGCCCAC (SEQ ID NO: 60);
GGCGCGGGTCATCTACGGGAC (SEQ ID NO: 61);
CCGCTGTGCCCGGCGACGCGG (SEQ ID NO: 62;
CCGCCCTTGC
AATCTGCGCCGGGCGTTTCAC (SEQ ID NO: 63);
CCGGCTCCGGC

GCGCCCGGTGTCCGGACGGCG (SEQ ID NO: 64);
CCGCCGGCGT
GGTTTCCCGGTCCGGCAAAGA (SEQ ID NO: 65);
AGAGGGCGCGG
GTGAACCGTCAGATCGCCTGG (SEQ ID NO: 66);
CTTGACACGATGGAGTCCTC (SEQ ID NO: 67);
GCCAAGAGTGACGTAAGTACC (SEQ ID NO: 68);
GTCTTTTCTGCAGTCACCGTC (SEQ ID NO: 69);
CAAGGTGCCACGGTACGTGTC (SEQ ID NO: 70);
CATGTGTTTAGGCCCGAGAC (SEQ ID NO: 71);
GGCAGAACTCGGTAAGTCTG (SEQ ID NO: 72);
CCTCCTCTACAGTCAAACAG (SEQ ID NO: 73);
GCGCCTATCATGCTGCCCCTC (SEQ ID NO: 74);
GCTCTCCCAGATGAACCACCC (SEQ ID NO: 75);
CAAGATTGACGAGGTGAGCCG (SEQ ID NO: 76);
CCCAAACAGGTCATGGTGCGC (SEQ ID NO: 77);
GCGTAAGAAACCGCGCAAAAC (SEQ ID NO: 78); and
CGCAAGAAGAAGAGCAAACGC (SEQ ID NO: 79).

3. The oligomer of claim 1 wherein the wherein the sequence of the oligomer is selected from the group consisting of:

GGGTTGAAAAACATAGCGGAC (SEQ ID NO: 1);
GAGGACTCCATCGTGTCAAG (SEQ ID NO: 2);
GTGGGCCATGATGATGGAAGG (SEQ ID NO: 3);
GTCCCGTAGATGACCCGCGCC (SEQ ID NO: 4);
CGGCGCAGATTGCAAGGGCGG (SEQ ID NO: 5);
GCCGGAGCCGGGTGAAACGCC (SEQ ID NO: 6);
CGCCGTCCGGACACCGGGCGC (SEQ ID NO: 7);
CCGGGAAACCACGCCGGCGG (SEQ ID NO: 8);
CCGCGCCCTCTTCTTTGCCGG (SEQ ID NO: 9);
GGTACTTACGTCACTCTTGGC (SEQ ID NO: 10);
GACGGTGACTGCAGAAAAGAC (SEQ ID NO: 11);
GACACGTACCGTGGCACCTTG (SEQ ID NO: 12);
GTCTCGGGCCTAAACACATG (SEQ ID NO: 13);
CAGACTTACCGACTTCTGCC (SEQ ID NO: 14);
CTGTTTGACTGTAGAGGAGG (SEQ ID NO: 15);
GGGTCCTTCATCTGGGAGAGC (SEQ ID NO: 16);

CGGCTCACCTCGTCAATCTTG (SEQ ID NO: 17);
GCGCACCATGACCTGTTTGGG (SEQ ID NO: 18);
CGTCTCCAGGCGATCTGACGC (SEQ ID NO: 19);
TGGCGTCTCCAGGCGATCTGA (SEQ ID NO: 20);
TGGCGTCTCCAGGCGATCTGA-K (SEQ ID NO: 21);
GTTTTGCGCGGTTTCTTACGC (SEQ ID NO: 22);
GCGTTTGCTCTTCTTCTTGCG (SEQ ID NO: 23);
GTTTGCTCTTCTTCTTG (SEQ ID NO: 24);
CGTTTGCTCTTCTTCTTGC (SEQ ID NO: 25);
GCGTTTTCTCTTCTGCTTGCG (SEQ ID NO: 26);
TCGGTTTCTCGTCTGCTTTCG (SEQ ID NO: 27);
GCGGTTTCTCTTCTGCTTTCG (SEQ ID NO: 28);
TATGGAGGTCAAAACAGCGTG (SEQ ID NO: 29);
TGGATCGGTCCCGGTGTCTTC (SEQ ID NO: 30);
ACCGTTCCCGGCCGCGGAGGC (SEQ ID NO: 31);
GGGGAATCCGCGTTCCAATGC (SEQ ID NO: 32);
CACCCGCGACCGCACCGCCGG (SEQ ID NO: 33);
CAGATACGGGTTGAAAAACAT (SEQ ID NO: 34);
TGGTGTAAGGCGGAGCCGCCG (SEQ ID NO: 35);
TGGTGTAAGGCGGGGCCGCCG (SEQ ID NO: 36);
CAGACGGGCCAGGGCCAGAAG (SEQ ID NO: 37);
CAGACGGGCCGGGGCCAGAAG (SEQ ID NO: 38);
TCCTGCGTGCCAGTCTGTCCG (SEQ ID NO: 39);
GTAGCCGTTTTTGCGATGTCG (SEQ ID NO: 40);
CCTCCTGGTTCAGACGTTCTC (SEQ ID NO: 41);
CAGTTTAACCCCGTATATCAC (SEQ ID NO: 42);
CAGCTTACGAAGCAAAATCAC (SEQ ID NO: 43);
CATAGCGGACCGTGAGAGGCT (SEQ ID NO: 44);
CATAGCGGACCGTGGGAGGCT (SEQ ID NO: 45);
CATAGCGGACCGTGAGGGGCT (SEQ ID NO: 46);
CATAGCGGACCGTGGGGGGCT (SEQ ID NO: 47);
AAACCCACGGCGGGGCTGTGT (SEQ ID NO: 48);
CGCGCGATGGCCCCGGCCTGC (SEQ ID NO: 49);
GCGTTTGCTCTTCTTCTTGC (SEQ ID NO: 50);
CGTTTGCTCTTCTTCTTGCG (SEQ ID NO: 51);
GCGTTTGCTCTTCTTCTTG (SEQ ID NO: 52);
GCGTTTGCTCTTCTTCTT (SEQ ID NO: 53);

GCGTTTGCTCTTCTTCT (SEQ ID NO: 54);
GTTTGCTCTTCTTCTTGCG (SEQ ID NO: 55);
TTTGCTCTTCTTCTTGCG (SEQ ID NO: 56); and
TTGCTCTTCTTCTTGCG (SEQ ID NO: 57).

4. The oligomer of claim 1 wherein substantially all the subunits of the oligomer are peptide nucleic acid subunits.

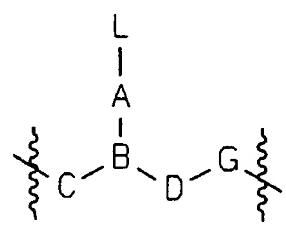
5. The oligomer of claim 2 incorporated in a pharmaceutically acceptable carrier.

6. An oligomer having a sequence selected from the group consisting of:
GGGTTGAAAAACATAGCGGAC (SEQ ID NO: 1);
GAGGACTCCATCGTGTCAAG (SEQ ID NO: 2);
GTGGGCCATGATGATGGAAGG (SEQ ID NO: 3);
GTCCCGTAGATGACCCGCGCC (SEQ ID NO: 4);
CGGCGCAGATTGCAAGGGCGG (SEQ ID NO: 5);
GCCGGAGCCGGGTGAAACGCC (SEQ ID NO: 6);
CGCCGTCCGGACACCGGGCGC (SEQ ID NO: 7);
CCGGGAAACCACGCCGGCGG (SEQ ID NO: 8);
CCGCGCCCTCTTCTTTGCCGG (SEQ ID NO: 9);
GGTACTTACGTCACTCTTGGC (SEQ ID NO: 10);
GACGGTGACTGCAGAAAAGAC (SEQ ID NO: 11);
GACACGTACCGTGGCACCTTG (SEQ ID NO: 12);
GTCTCGGGCCTAAACACATG (SEQ ID NO: 13);
CAGACTTACCGACTTCTGCC (SEQ ID NO: 14);
CTGTTTGACTGTAGAGGAGG (SEQ ID NO: 15);
GGGTCCTTCATCTGGGAGAGC (SEQ ID NO: 16);
CGGCTCACCTCGTCAATCTTG (SEQ ID NO: 17);
GCGCACCATGACCTGTTTGGG (SEQ ID NO: 18);
CGTCTCCAGGCGATCTGACGC (SEQ ID NO: 19);
TGGCGTCTCCAGGCGATCTGA (SEQ ID NO: 20);
TGGCGTCTCCAGGCGATCTGA-K (SEQ ID NO: 21);
GTTTTGCGCGGTTTCTTACGC (SEQ ID NO: 22);
GCGTTTGCTCTTCTTCTTGCG (SEQ ID NO: 23);

GTTTGCTCTTCTTCTTG (SEQ ID NO: 24);
CGTTTGCTCTTCTTCTTGC (SEQ ID NO: 25);
GCGTTTTCTCTTCTGCTTGCG (SEQ ID NO: 26);
TCGGTTTCTCGTCTGCTTTCG (SEQ ID NO: 27);
GCGGTTTCTCTTCTGCTTTCG (SEQ ID NO: 28);
TATGGAGGTCAAAACAGCGTG (SEQ ID NO: 29);
TGGATCGGTCCCGGTGTCTTC (SEQ ID NO: 30);
ACCGTTCCCGGCCGCGGAGGC (SEQ ID NO: 31);
GGGGAATCCGCGTTCCAATGC (SEQ ID NO: 32);
CACCCGCGACCGCACCGCCGG (SEQ ID NO: 33);
CAGATACGGGTTGAAAAACAT (SEQ ID NO: 34);
TGGTGTAAGGCGGAGCCGCCG (SEQ ID NO: 35);
TGGTGTAAGGCGGGGCCGCCG (SEQ ID NO: 36);
CAGACGGGCCAGGGCCAGAAG (SEQ ID NO: 37);
CAGACGGGCCGGGGCCAGAAG (SEQ ID NO: 38);
TCCTGCGTGCCAGTCTGTCCG (SEQ ID NO: 39);
GTAGCCGTTTTTGCGATGTCG (SEQ ID NO: 40);
CCTCCTGGTTCAGACGTTCTC (SEQ ID NO: 41);
CAGTTTAACCCCGTATATCAC (SEQ ID NO: 42);
CAGCTTACGAAGCAAAATCAC (SEQ ID NO: 43);
CATAGCGGACCGTGAGAGGCT (SEQ ID NO: 44);
CATAGCGGACCGTGGGAGGCT (SEQ ID NO: 45);
CATAGCGGACCGTGAGGGGCT (SEQ ID NO: 46);
CATAGCGGACCGTGGGGGGCT (SEQ ID NO: 47);
AAACCCACGGCGGGGCTGTGT (SEQ ID NO: 48);
CGCGCGATGGCCCCGGCCTGC (SEQ ID NO: 49);
GCGTTTGCTCTTCTTCTTGC (SEQ ID NO: 50);
CGTTTGCTCTTCTTCTTGCG (SEQ ID NO: 51);
GCGTTTGCTCTTCTTCTTG (SEQ ID NO: 52);
GCGTTTGCTCTTCTTCTT (SEQ ID NO: 53);
GCGTTTGCTCTTCTTCT (SEQ ID NO: 54);
GTTTGCTCTTCTTCTTGCG (SEQ ID NO: 55);
TTTGCTCTTCTTCTTGCG (SEQ ID NO: 56);
TTGCTCTTCTTCTTGCG (SEQ ID NO: 57);
GGACCGGGACCACCGTCGTC (SEQ ID NO: 58);
GTCCGCTATGTTTTTCAACCC (SEQ ID NO: 59);
CCTTCCATCATCATGGCCCAC (SEQ ID NO: 60);

GGCGCGGGTCATCTACGGGAC (SEQ ID NO: 61);
CCGCTGTGCCCGGCGACGCGG (SEQ ID NO: 62;
CCGCCCTTGC
AATCTGCGCCGGGCGTTTCAC (SEQ ID NO: 63);
CCGGCTCCGGC
GCGCCCGGTGTCCGGACGGCG (SEQ ID NO: 64);
CCGCCGGCGT
GGTTTCCCGGTCCGGCAAAGA (SEQ ID NO: 65);
AGAGGGCGCGG
GTGAACCGTCAGATCGCCTGG (SEQ ID NO: 66);
CTTGACACGATGGAGTCCTC (SEQ ID NO: 67);
GCCAAGAGTGACGTAAGTACC (SEQ ID NO: 68);
GTCTTTTCTGCAGTCACCGTC (SEQ ID NO: 69);
CAAGGTGCCACGGTACGTGTC (SEQ ID NO: 70);
CATGTGTTTAGGCCCGAGAC (SEQ ID NO: 71);
GGCAGAACTCGGTAAGTCTG (SEQ ID NO: 72);
CCTCCTCTACAGTCAAACAG (SEQ ID NO: 73);
GCGCCTATCATGCTGCCCCTC (SEQ ID NO: 74);
GCTCTCCCAGATGAACCACCC (SEQ ID NO: 75);
CAAGATTGACGAGGTGAGCCG (SEQ ID NO: 76);
CCCAAACAGGTCATGGTGCGC (SEQ ID NO: 77);
GCGTAAGAAACCGCGCAAAAC (SEQ ID NO: 78); and
CGCAAGAAGAAGAGCAAACGC (SEQ ID NO: 79); and

wherein at least one subunit of the oligomer is a peptide nucleic acid subunit of the formula:



(I)

wherein:

L is one of the adenine, thymine, cytosine or guanine heterocyclic bases of the oligomer;

C is $(CR^6R^7)_y$ where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently

selected from the group consisting of hydrogen, (C_1-C_6)alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C_1-C_6)alkoxy, (C_1-C_6)alkylthio, NR^3R^4 and SR^5, where each of R^3 and R^4 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl, hydroxy- or alkoxy- or alkylthio-substituted (C_1-C_4)alkyl, hydroxy, alkoxy, alkylthio and amino;, and R^5 is hydrogen, (C_1-C_6)alkyl, hydroxy-, alkoxy-, or alkylthio-substituted (C_1-C_6)alkyl, or R^6 and R^7 taken together complete an alicyclic or heterocyclic system;

D is $(CR^6R^7)_z$ where R^6 and R^7 are as defined above;

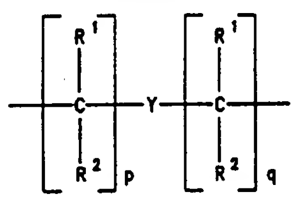
each of y and z is zero or an integer from 1 to 10, the sum y + z being greater than 2 but not more than 10;

G is $-NR^3CO-$, $-NR^3CS-$, $-NR^3SO-$ or $-NR^3SO_2-$, in either orientation, where R^3 is as defined above;

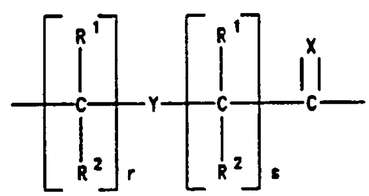
each pair of A and B is selected such that:

(a) A is a group of formula (IIa), (IIb) or (IIc) and B is N or R^3N^+; or

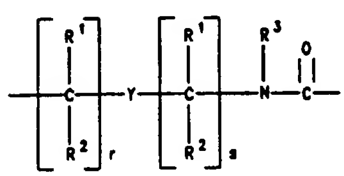
(b) A is a group of formula (IId) and B is CH;



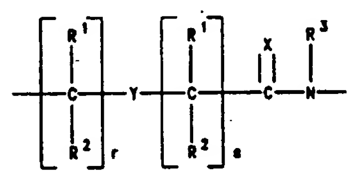
(IIa)



(IIb)



(IIc)



(IId)

where:

X is O, S, Se, NR^3, CH_2 or $C(CH_3)_2$;

Y is a single bond, O, S or NR^4;

each of p and q is zero or an integer from 1 to 5, the sum p+q being not more than 10;

each of r and s is zero or an integer from 1 to 5, the sum r+s being not more than 10;

each R^1 and R^2 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl which may be hydroxy- or alkoxy- or alkylthio-substituted, hydroxy, alkoxy, alkylthio, amino and halogen.

7. The oligomer of claim 6 wherein A is $-CH_2CO-$, B is N, C is CH_2CH_2 and D is CH_2.

8. The oligomer of claim 6 wherein all of the subunits are peptide nucleic acid subunits;

said oligomer including a group Q on one end of said oligomer and a group I on the other end of said oligomer;

Q is $-CO_2H$, $-CONR'R''$, $-SO_3H$ or $-SO_2NR'R''$ or an activated derivative of $-CO_2H$ or $-SO_3H$; and

I is $-NHR'''R''''$ or $-NR'''C(O)R''''$, where R', R", R''' and R'''' are independently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, oligonucleotides and soluble and non-soluble polymers.

9. An oligomer hybridizable to the E, E2, E4, E5, E6, E7, L1 or L2 reading frames of a papillomavirus and comprising at least one peptide nucleic acid subunit.

10. The oligomer of claim 9 wherein the sequence of the oligomer is selected from the group consisting of:

AGGTTTGCACCCGACTATGCAAGTACAAAT (SEQ ID NO: 80);
TATGCAAGTACAAAT (SEQ ID NO: 81);
CGTTCGCATGCTGTCTCCATCCTCTTCACT (SEQ ID NO: 82);
GCATGCTGTCTCCAT (SEQ ID NO: 83);
AAATGCGTCCAGCACCGGCCATGGTGCAGT (SEQ ID NO: 84);
AGCACCGGCCATGGT (SEQ ID NO: 85);
CAATGGCAGTGATCAGAAGTCCAAGCTGGC (SEQ ID NO: 86);
GCAGTGATCAGAAGT (SEQ ID NO: 87);

ATTGCTGCAGCTTAAACCATATAAAATCTG (SEQ ID NO: 88);
CTTAAACCATATAAA (SEQ ID NO: 89);
AAAAAAAGATTTCCAATCTGCATCAGTAAT (SEQ ID NO: 90);
AAGATTTCCAATCTG (SEQ ID NO: 91);
CAGTGTCCTAGGACAGTCACCCCTTTTTTC (SEQ ID NO: 92);
GGACAGTCACCCCTT (SEQ ID NO: 93);
TGTACAAATTGCTGTAGACAGTGTACCAGT (SEQ ID NO: 94);
GCTGTAGACAGTGTA (SEQ ID NO: 95);
GTGCGAGCGAGGACCGTCCCGTACCCAACC (SEQ ID NO: 96);
GGACCGTCCCGTACC (SEQ ID NO: 97);
TTTAACAGGTGGAATCCATCATTGGTGGTG (SEQ ID NO: 98);
GGAATCCATCATTGG (SEQ ID NO: 99);
GCTTCCATCTTCCTC (SEQ ID NO: 100);
GCTTCCATCTTCCTCG (SEQ ID NO: 101);
TGCTTCCATCTTCCTCG (SEQ ID NO: 102);
TGCTTCCATCTTCCTCGT (SEQ ID NO: 103);
TTGCTTCCATCTTCCTCGT (SEQ ID NO: 104);
TTGCTTCCATCTTCCTCGTC (SEQ ID NO: 105);
CGACTATGCAAGTAC (SEQ ID NO: 106);
CGACTATGCAATTTC (SEQ ID NO: 107);
TTTCTATGCAAGTAC (SEQ ID NO: 108);
CGACTATGCAACCCC (SEQ ID NO: 109);
TCTCCATCCTCTTCACT (SEQ ID NO: 110);
AGCGCGCCATAGTATTGTGG (SEQ ID NO: 111);
GTCCATGCATACTTAATATT (SEQ ID NO: 112);
TATTACGTACTAGATTCTAC (SEQ ID NO: 113); and
CTGTCTCCATCCTCTTCACT (SEQ ID NO: 114).

11. The oligomer of claim 9 wherein the sequence of the oligomer is selected from the group consisting of:
AGGTTTGCACCCGACTATGCAAGTACAAAT (SEQ ID NO: 80);
CGTTCGCATGCTGTCTCCATCCTCTTCACT (SEQ ID NO: 82);
GGACAGTCACCCCTT (SEQ ID NO: 93);
TTGCTTCCATCTTCCTCGTC (SEQ ID NO: 105);
AGCGCGCCATAGTATTGTGG (SEQ ID NO: 110); and
GTCCATGCATACTTAATATT (SEQ ID NO: 112).

12. The oligomer of claim 9 wherein substantially all the subunits of the oligomer are peptide nucleic acid subunits.

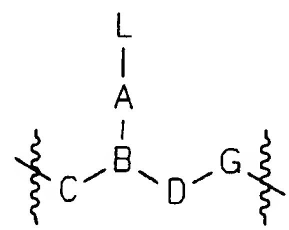
13. The oligomer of claim 10 incorporated in a pharmaceutically acceptable carrier.

14. An oligomer having a sequence selected from the group consisting of:

AGGTTTGCACCCGACTATGCAAGTACAAAT (SEQ ID NO: 80);
TATGCAAGTACAAAT (SEQ ID NO: 81);
CGTTCGCATGCTGTCTCCATCCTCTTCACT (SEQ ID NO: 82);
GCATGCTGTCTCCAT (SEQ ID NO: 83);
AAATGCGTCCAGCACCGGCCATGGTGCAGT (SEQ ID NO: 84);
AGCACCGGCCATGGT (SEQ ID NO: 85);
CAATGGCAGTGATCAGAAGTCCAAGCTGGC (SEQ ID NO: 86);
GCAGTGATCAGAAGT (SEQ ID NO: 87);
ATTGCTGCAGCTTAAACCATATAAAATCTG (SEQ ID NO: 88);
CTTAAACCATATAAA (SEQ ID NO: 89);
AAAAAAAGATTTCCAATCTGCATCAGTAAT (SEQ ID NO: 90);
AAGATTTCCAATCTG (SEQ ID NO: 91);
CAGTGTCCTAGGACAGTCACCCCTTTTTTC (SEQ ID NO: 92);
GGACAGTCACCCCTT (SEQ ID NO: 93);
TGTACAAATTGCTGTAGACAGTGTACCAGT (SEQ ID NO: 94);
GCTGTAGACAGTGTA (SEQ ID NO: 95);
GTGCGAGCGAGGACCGTCCCGTACCCAACC (SEQ ID NO: 96);
GGACCGTCCCGTACC (SEQ ID NO: 97);
TTTAACAGGTGGAATCCATCATTGGTGGTG (SEQ ID NO: 98);
GGAATCCATCATTGG (SEQ ID NO: 99);
GCTTCCATCTTCCTC (SEQ ID NO: 100);
GCTTCCATCTTCCTCG (SEQ ID NO: 101);
TGCTTCCATCTTCCTCG (SEQ ID NO: 102);
TGCTTCCATCTTCCTCGT (SEQ ID NO: 103);
TTGCTTCCATCTTCCTCGT (SEQ ID NO: 104);
TTGCTTCCATCTTCCTCGTC (SEQ ID NO: 105);
CGACTATGCAAGTAC (SEQ ID NO: 106);
CGACTATGCAATTTC (SEQ ID NO: 107);

TTTCTATGCAAGTAC (SEQ ID NO: 108);
CGACTATGCAACCCC (SEQ ID NO: 109);
TCTCCATCCTCTTCACT (SEQ ID NO: 110);
AGCGCGCCATAGTATTGTGG (SEQ ID NO: 111);
GTCCATGCATACTTAATATT (SEQ ID NO: 112);
TATTACGTACTAGATTCTAC (SEQ ID NO: 113); and
CTGTCTCCATCCTCTTCACT (SEQ ID NO: 114); and

wherein at least one subunit of the oligomer is a peptide nucleic acid subunit of the formula:



(I)

wherein:

L is one of the adenine, thymine, cytosine or guanine heterocyclic bases of the oligomer;

C is $(CR^6R^7)_y$ where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently selected from the group consisting of hydrogen, (C_2-C_6)alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C_1-C_6)alkoxy, (C_1-C_6)alkylthio, NR^3R^4 and SR^5, where each of R^3 and R^4 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl, hydroxy- or alkoxy- or alkylthio-substituted (C_1-C_4)alkyl, hydroxy, alkoxy, alkylthio and amino;, and R^5 is hydrogen, (C_1-C_6)alkyl, hydroxy-, alkoxy-, or alkylthio-substituted (C_1-C_6)alkyl, or R^6 and R^7 taken together complete an alicyclic or heterocyclic system;

D is $(CR^6R^7)_z$ where R^6 and R^7 are as defined above;

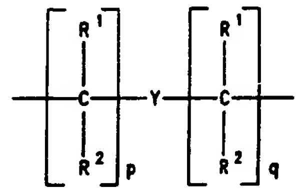
each of y and z is zero or an integer from 1 to 10, the sum y + z being greater than 2 but not more than 10;

G is $-NR^3CO-$, $-NR^3CS-$, $-NR^3SO-$ or $-NR^3SO_2-$, in either orientation, where R^3 is as defined above;

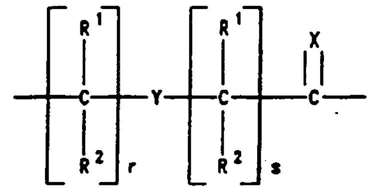
each pair of A and B is selected such that:

(a) A is a group of formula (IIa), (IIb) or (IIc) and B is N or R^3N^+; or

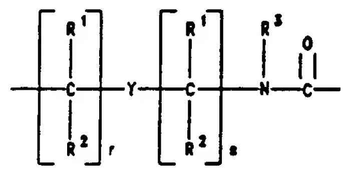
(b) A is a group of formula (IId) and B is CH;



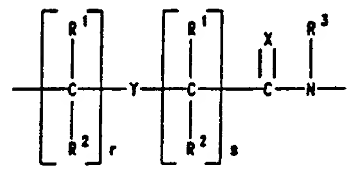
(IIa)



(IIb)



(IIc)



(IId)

where:

X is O, S, Se, NR^3, CH_2 or $C(CH_3)_2$;

Y is a single bond, O, S or NR^4;

each of p and q is zero or an integer from 1 to 5, the sum p+q being not more than 10;

each of r and s is zero or an integer from 1 to 5, the sum r+s being not more than 10;

each R^1 and R^2 is independently selected from the group consisting of hydrogen, $(C_1\text{-}C_4)$alkyl which may be hydroxy- or alkoxy- or alkylthio-substituted, hydroxy, alkoxy, alkylthio, amino and halogen.

15. The oligomer of claim 14 wherein A is $-CH_2CO-$, B is N, C is CH_2CH_2 and D is CH_2.

16. The oligomer of claim 14 wherein all of the subunits are peptide nucleic acid subunits;

said oligomer including a group Q on one end of said oligomer and a group I on the other end of said oligomer;

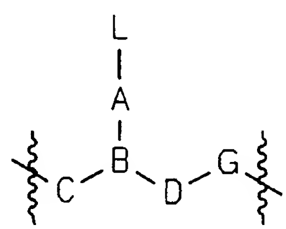
Q is $-CO_2H$, $-CONR'R''$, $-SO_3H$ or $-SO_2NR'R''$ or an activated derivative of $-CO_2H$ or $-SO_3H$; and

I is -NHR'''R'''' or -NR'''C(O)R'''', where R', R", R''' and R'''' are independently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, oligonucleotides and soluble and non-soluble polymers.

17. A method of modulating a viral process comprising contacting a cell with an oligomer comprising a sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 114 and having at least one peptide nucleic acid subunit.

18. The method of claim 17 wherein the substantially all of the subunits of the oligomer are peptide nucleic acid subunits.

19. A method of modulating a viral process comprising contacting a cell with an oligomer comprising a sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 114 wherein at least one subunit of the oligomer is a peptide nucleic acid subunit of the formula:



(I)

wherein:

L is one of the adenine, thymine, cytosine or guanine heterocyclic bases of the oligomer;

C is $(CR^6R^7)_y$ where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently selected from the group consisting of hydrogen, (C_2-C_6)alkyl,

aryl, aralkyl, heteroaryl, hydroxy, (C_1-C_6)alkoxy, (C_1-C_6)alkylthio, NR^3R^4 and SR^5, where each of R^3 and R^4 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl, hydroxy- or alkoxy- or alkylthio-substituted (C_1-C_4)alkyl, hydroxy, alkoxy, alkylthio and amino;, and R^5 is hydrogen, (C_1-C_6)alkyl, hydroxy-, alkoxy-, or alkylthio-substituted (C_1-C_6)alkyl, or R^6 and R^7 taken together complete an alicyclic or heterocyclic system;

D is $(CR^6R^7)_z$ where R^6 and R^7 are as defined above;

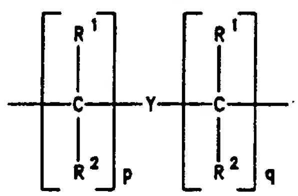
each of y and z is zero or an integer from 1 to 10, the sum y + z being greater than 2 but not more than 10;

G is $-NR^3CO-$, $-NR^3CS-$, $-NR^3SO-$ or $-NR^3SO_2-$, in either orientation, where R^3 is as defined above;

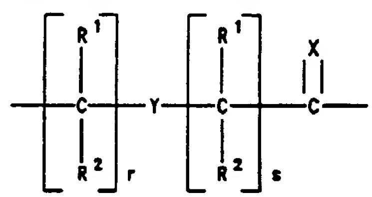
each pair of A and B is selected such that:

(a) A is a group of formula (IIa), (IIb) or (IIc) and B is N or R^3N^+; or

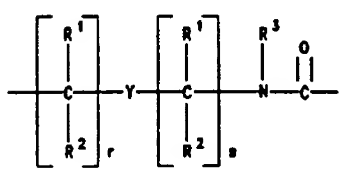
(b) A is a group of formula (IId) and B is CH;



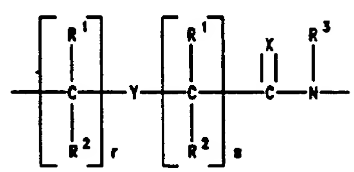
(IIa)



(IIb)



(IIc)



(IId)

where:

X is O, S, Se, NR^3, CH_2 or $C(CH_3)_2$;

Y is a single bond, O, S or NR^4;

each of p and q is zero or an integer from 1 to 5, the sum p+q being not more than 10;

each of r and s is zero or an integer from 1 to 5, the sum r+s being not more than 10;

each R^1 and R^2 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl which may be hydroxy- or alkoxy- or alkylthio-substituted, hydroxy, alkoxy, alkylthio, amino and halogen.

20. The method of claim 19 wherein A is $-CH_2CO-$, B is N, C is CH_2CH_2 and D is CH_2.

21. The oligomer of claim 20 wherein all of the subunits are peptide nucleic acid subunits;

said oligomer including a group Q on one end of said oligomer and a group I on the other end of said oligomer;

Q is $-CO_2H$, $-CONR'R''$, $-SO_3H$ or $-SO_2NR'R''$ or an activated derivative of $-CO_2H$ or $-SO_3H$; and

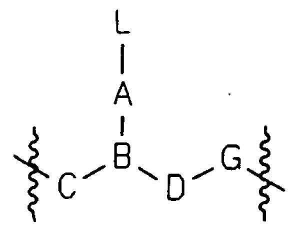
I is $-NHR'''R''''$ or $-NR'''C(O)R''''$, where R', R", R''' and R'''' are independently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, oligonucleotides and soluble and non-soluble polymers.

22. A method of treating a mammal having a disease characterized by a viral infection comprising admininstering to said mammal an oligomer comprising a sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 114 and having at least one peptide nucleic acid subunit.

23. The method of claim 22 wherein the substantially all of the subunits of the oligomer are peptide nucleic acid subunits.

24. A method of treating a mammal having a disease characterized by a viral infection comprising admininstering to said mammal an oligomer comprising a sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO:

114 wherein at least one subunit of the oligomer is a peptide nucleic acid subunit of the formula:



(I)

wherein:

L is one of the adenine, thymine, cytosine or guanine heterocyclic bases of the oligomer;

C is $(CR^6R^7)_y$ where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently selected from the group consisting of hydrogen, (C_2-C_6)alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C_1-C_6)alkoxy, (C_1-C_6)alkylthio, NR^3R^4 and SR^5, where each of R^3 and R^4 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl, hydroxy- or alkoxy- or alkylthio-substituted (C_1-C_4)alkyl, hydroxy, alkoxy, alkylthio and amino;, and R^5 is hydrogen, (C_1-C_6)alkyl, hydroxy-, alkoxy-, or alkylthio-substituted (C_1-C_6)alkyl, or R^6 and R^7 taken together complete an alicyclic or heterocyclic system;

D is $(CR^6R^7)_z$ where R^6 and R^7 are as defined above;

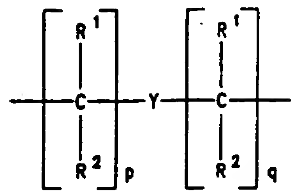
each of y and z is zero or an integer from 1 to 10, the sum y + z being greater than 2 but not more than 10;

G is $-NR^3CO-$, $-NR^3CS-$, $-NR^3SO-$ or $-NR^3SO_2-$, in either orientation, where R^3 is as defined above;

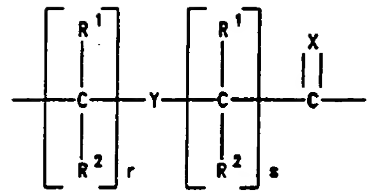
each pair of A and B is selected such that:

(a) A is a group of formula (IIa), (IIb) or (IIc) and B is N or R^3N^+; or

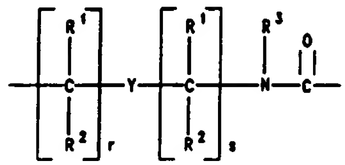
(b) A is a group of formula (IId) and B is CH;



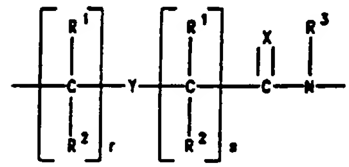
(IIa)



(IIb)



(IIc)



(IId)

where:

X is O, S, Se, NR^3, CH_2 or $C(CH_3)_2$;

Y is a single bond, O, S or NR^4;

each of p and q is zero or an integer from 1 to 5, the sum p+q being not more than 10;

each of r and s is zero or an integer from 1 to 5, the sum r+s being not more than 10;

each R^1 and R^2 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl which may be hydroxy- or alkoxy- or alkylthio-substituted, hydroxy, alkoxy, alkylthio, amino and halogen.

25. The method of claim 24 wherein A is $-CH_2CO-$, B is N, C is CH_2CH_2 and D is CH_2.

26. The oligomer of claim 24 wherein all of the subunits are peptide nucleic acid subunits;

said oligomer including a group Q on one end of said oligomer and a group I on the other end of said oligomer;

Q is $-CO_2H$, $-CONR'R''$, $-SO_3H$ or $-SO_2NR'R''$ or an activated derivative of $-CO_2H$ or $-SO_3H$; and

I is $-NHR'''R''''$ or $-NR'''C(O)R''''$, where R', R", R''' and R'''' are independently selected from the group

consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, oligonucleotides and soluble and non-soluble polymers.

INTERNATIONAL SEARCH REPORT

International application No. PCT/US94/09039

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07H 21/00, 21/02, 21/04; A61K 31/73, 31/74, 31/765, 31/785
US CL :514/44; 536/22.1, 24.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 536/22.1, 23.1, 24.1, 24.31, 24.5, 25.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS, BIOSIS, APS, MEDLINE, BIOTECH ABS.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, A, 92/20702 (BUCHARDT ET AL.) 26 NOVEMBER 1992, SEE CLAIMS 1-33 ON PAGES 145-156.	1-26
Y	JOURNAL OF MOLECULAR BIOLOGY, VOLUME 193, ISSUED 1987, COLE ET AL., "NUCLEOTIDE SEQUENCE AND COMPARATIVE ANALYSIS OF THE HUMAN PAPILLOMAVIRUS TYPE 18 GENOME", SEE ESPECIALLY THE ABSTRACT AND FIGURE 1 ON PAGES 601-603 AND FIGURE 2 ON PAGE 604.	9-26
Y	JOURNAL OF CELL BIOLOGY, SUPPLEMENT 0, VOLUME 17 PART E, ISSUED 12-18 APRIL 1993, VICKERS ET AL., SITE SPECIFIC TERMINATION OF TRANSCRIPTION IN EUKARYOTIC CELL EXTRACTS BY PEPTIDE NUCLEIC ACID (PNA) OLIGOMERS", PAGE 215, ABSTRACT NO. S 418, SEE ENTIRE ABSTRACT.	1-26

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 SEPTEMBER 1994	17 OCT 1994

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	ARDIN MARSCHEL D. Kuza for
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCIENCE, VOLUME 254, ISSUED 06 DECEMBER 1991, NIELSEN ET AL., "SEQUENCE-SELECTIVE RECOGNITION OF DNA BY STRAND DISPLACEMENT WITH THYMINE-SUBSTITUTED POLYAMIDE", PAGES 1497-1500, SEE ESPECIALLY THE ABSTRACT AND FIGURE 1 ON PAGE 1498 AND FIGURE 4 ON PAGE 1499.	1-26
Y	VIROLOGY, VOLUME 114, ISSUED 1981, DEMARCHI, "HUMAN CYTOMEGALOVIRUS DNA: RESTRICTION ENZYME CLEAVAGE MAPS AND MAP LOCATIONS FOR IMMEDIATE-EARLY, EARLY, AND LATE RNAs", PAGES 23-38, SEE ESPECIALLY THE ABSTRACT AND FIGURE 7 ON PAGE 35.	1-8 AND 17-26
Y	JOURNAL OF VIROLOGY, VOLUME 42, NUMBER 2, ISSUED MAY 1982, TAMASHIRO ET AL., "CONSTRUCTION OF A CLONED LIBRARY OF THE *ECO*RI FRAGMENTS FROM THE HUMAN CYTOMEGALOVIRUS GENOME (STRAIN AD169)", PAGES 547-557, SEE ESPECIALLY THE ABSTRACT AND TABLE 1 ON PAGE 549.	1-8 AND 17-26